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A NEW SPECIES OF *HENDERSONULA* (*H. TORULOIDEA*) ON DECIDUOUS TREES IN EGYPT

By R. M. NATTRASS
(*Cyprus*)

(With Plates IX, X and 5 Text-figures)

Introduction

One of the most serious diseases to which stone fruit trees in Egypt are subject is a form of Gummosis and Die-Back. Early symptoms in mature trees are a wilting and "die-back" of twigs and branches, accompanied by a copious exudation of a gum-like substance; the tree itself may succumb slowly, one branch being affected followed later by others. Though the tree may survive one or two seasons, the whole system eventually becomes involved. In other instances trees in their prime and in full bearing may suddenly wilt, beads of the gum-like substance are exuded from the lenticels and from the bases of the buds and leaf trusses, and death quickly ensues.

During an investigation of this disease an attempt was made to isolate a causal organism, and numerous isolations were made from diseased tissue from all aerial parts of affected trees. These isolations invariably yielded a dark-spored Hyphomycete, usually uncontaminated with any other fungus. Material of diseased stone fruit trees from many orchards from the Delta to Minia were examined and in every instance the fungus appeared in culture, but no fructifications were seen in nature during the initial investigation.

The investigation was extended to the "die-back" disease of apple trees, which do not as a rule flourish in Upper Egypt, and showed that the fungus also occurs on this host but to a less extent than on stone fruits. In no instance was the fungus isolated from citrus trees.

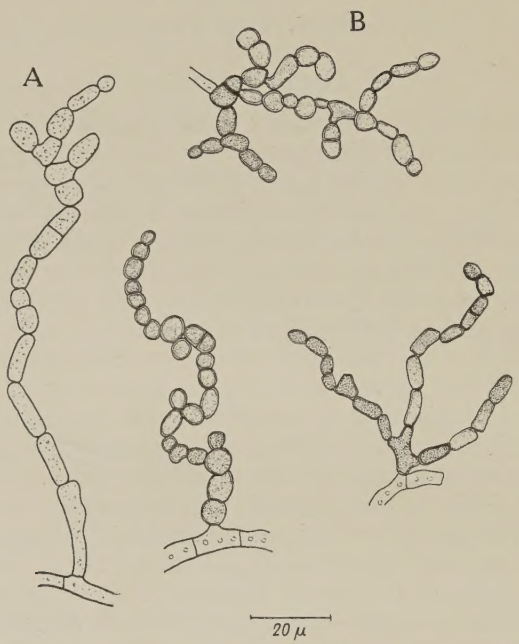
General observations on the incidence of the disease on stone fruit trees indicated that the fungus was not a primary parasite and that the initial symptom of gumming was the manifestation of physiological disturbances brought about by unsuitable soil conditions and water-logging due either to over-irrigation or to fluctuation of the water table, with inadequate drainage, following a high Nile.

The present paper is concerned solely with the fungus which appears to be universally associated with the disease in Egypt and which, as will appear, may produce a Sphaeropsid form in its life history.

The *Torula* stage in culture

Isolations from the diseased tissue of the affected twigs and branches almost invariably yielded a pure culture of a *Torula* form (Pl. IX, fig. 1). The fungus grows readily on prune extract agar at 30° C. After forty-eight hours a white pulverulent growth is produced with minute knots of aerial mycelium just visible to the naked eye (Pl. IX, fig. 2).

After the third or fourth day the culture darkens in colour, becoming a dusky olive-green*, with a white pulverulent margin;



Text-fig. 1. *Torula* stage in culture. A, hyphae not fully fragmented into brown thallospores. B, same, fully fragmented.

the whole eventually becoming olivaceous black. The aerial hyphae and those on and below the substratum break up, within a short distance from their point of origin, into chains of thallospores. The appearance of these chains is characteristic, the effect being produced by the occurrence of frequent cells of a subcuneate form and two apices at which the branching of the chain originates (Text-fig. 1 A, B). The aerial chains of thallospores are considerably convoluted, appearing as minute tufts or knots visible to the naked eye.

* Colours are those of Ridgway, R., *Color Standards and Nomenclature*, Washington, 1912.

The thallospores vary in shape from globose, 4μ in diameter, to oblong up to 10×4μ; the latter being occasionally 1-septate. They are at first hyaline, becoming citrine drab and later, in old cultures, turning bone brown and developing a thick wall. Among them are numbers of the characteristic cuneiform cells.

Below the substratum the characteristic branching produces fan-shaped plates of mycelial tissue.

The optimum temperature of growth is between 30 and 35° C., but vigorous growth also occurs at 40° C.

Formation of the pycnidia in culture

No fructifications other than those of the *Torula* form ever appeared on the agar media. Pure cultures of the fungus were grown on sterilised blocks of cotton and willow twig in flasks and Roux tubes. On these media the *Torula* form grew vigorously, quickly covering the surface of the wood with the aerial mycelium. A characteristic feature of this growth on wood, in a saturated atmosphere, is the formation of black or grey spongy masses of mycelium dotted over the surface of the wood. In from one to four months at room temperature the production of black carbonaceous pycnidia was observed. These pycnidia occur singly or in small aggregates on the surface of the medium, partly immersed in the mass of aerial mycelium; there is a distinct subicle or rudimentary stroma in the aggregate form. They are spherical and measure from 120 to 195μ in diameter, with a short-beaked ostiole up to 70μ in length (Pl. IX, fig. 3).

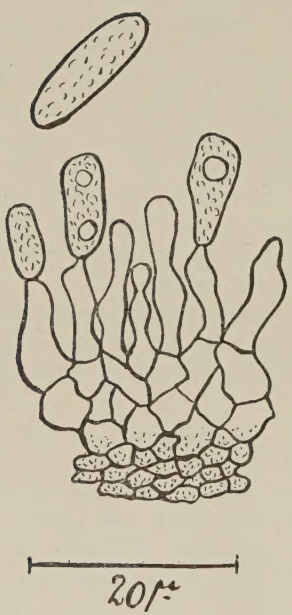
In a moist atmosphere, the pycnospores are extruded in the form of white tendrils or globular masses. When a group of pycnidia is placed in water the spores are ejected with almost explosive violence from the ostiole of the pycnidium.

Inside the pycnidium and when first extruded, the spores are hyaline or of a slight greenish tinge with granular contents. Immediately after extrusion from the pycnidium two or more spores may frequently be seen attached by their extremities in the form of a chain, apparently by the mucilaginous envelope enclosing them. Portions of this envelope can sometimes be seen on a single spore as a minute appendage.

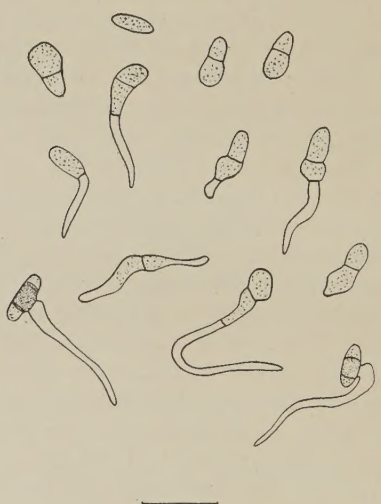
The pycnospores are borne on short, hyaline, somewhat tapering, slightly flexuous sporophores 8–10μ in length and up to 4μ in breadth (Text-fig. 3); they measure 12–13·5 (ex. 17μ) × 4–5·5μ (Text-fig. 2 A). These hyaline spores do not germinate in water at room temperature (16° C.) after forty-eight hours. They germinate readily, however, after twenty-four hours at 30° C. Prior to germination the spores increase considerably in size and usually become 1-septate and produce one germ-tube or more rarely two from the extremities (Text-fig. 4).

In hanging drops of prune agar after forty-eight hours at 30° C. mycelium from the germ-tube develops into the *Torula* stage with cells

Text-fig. 2. Pycnospores. A, hyaline. B, coloured.



Text-fig. 3.



Text-fig. 4.

Text-fig. 3. Sporophores and hyaline pycnospores *in situ*.

Text-fig. 4. Germinating pycnospores. Hyaline spores with median septum and apical germ-tube. Coloured spores with lateral germ-tube.

resembling the diversely shaped cells of the *Torula* stage from the original isolation. Monospore cultures on prune extract agar appeared identical with those from the original isolations.

These cultures in turn, when grown on wood blocks, eventually produced the *Phoma*-like pycnidia and hyaline spores identical with those of the original cultures.

The tendrils and globular masses of spores extruded from the pycnidia in the wood-block cultures, sooner or later (apparently depending on the amount of desiccation) become pitch black in mass. This is due to the fact that the hyaline spores develop a dark central cell, hence becoming 2-septate (Text-fig. 2 B). These dark spores do not come apart readily in water, neither do they germinate so readily as do the hyaline spores. They do not swell prior to germination and only the dark central cell produces a germ-tube, which consequently emerges laterally* (Text-fig. 4).

The germinating spore has a characteristic appearance due to the fact that the germ tube, at the point of emergence, is of a greater diameter than the spore itself.

Monospore cultures were made from these dark 2-septate pycnospores; these again produced the *Torula* form identical with that of the original isolations.

The dark-spored Hyphomycete is therefore shown to have a pycnidial stage, the spores of which do not become fully mature until after they have left the pycnidium.

The pycnidial stage in nature

As has already been stated, the fungus was cultured and studied for two years before any fructifications were found in nature. It was clear that, in order to function as a disseminating agent, the pycnidial fructifications must appear on the surface of the natural substratum.

Although plum, apricot, and apple trees suffering from "die-back" were examined periodically from 1927 to 1929 and the *Torula* stage had been invariably isolated from the diseased tissue, no actual fructifications, occurring on the natural substratum, were found till July, 1930. A piece of a branch of the natural apricot, showing symptoms of "die-back," was collected at Maadi and placed in a large plugged flask containing about 4 cm. depth of water and kept at room temperature (then approx. 25° C.). No external signs of fructifications were then visible, but after four to five weeks a few fructifications, visible to the naked eye, appeared scattered irregularly and sparsely on the surface of the bark. These eventually developed into large black erumpent stromata on which a number of partly immersed pycnidia could be distinguished with a hand lens (Pl. IX, fig. 4). A typical fructification measured $1 \times 0{\cdot}5$ mm. and projected

* It appears to be worth noting that the hyaline pycnospores, on germination, acquire an approximately median septum; whereas after the formation of the coloured cell this character is completely lost.

about 0·5 mm. above the surface of the bark. A stroma, when placed in a drop of water, produced masses of spores from the various ostioles.

The subsequent behaviour of these fructifications agreed with that of the pycnidia produced in the wood-block cultures; white tendrils were extruded and later masses of the dark 2-septate spores measuring 10·5–14 × 4·5–5·5 μ were found round the ostioles. Monospore cultures of the two forms of pycnospores both produced the *Torula* stage identical with that from the original isolations.

It was obvious that, under suitable conditions, the pycnidial fructifications must occur in nature and that mature fructifications would be revealed by further search. Trees in the same locality were examined during the ensuing weeks and eventually visible fructifications were found, which quickly matured in a moist atmosphere. From this material it was possible to determine more fully the nature of the stromata.

They originate in the cortex and, when mature, are remarkable for their comparatively large volume, a considerable portion of which may extend laterally below the part bearing the pycnidia. Great variation occurs in the shape and size of the stromata. They may be wholly immersed in the substratum, with the ostiole of a single large pycnidium breaking through the bark. Other immersed stromata may possess one or more smaller pycnidia formed subsequently, but at this stage usually only the large pycnidium has access to the air. Other stromata may be flush with the surface of the bark, eventually becoming erumpent. When in close proximity one or more stromata may be connected below the bark by a thick sclerotial plate (Pl. X, figs. 1–3).

Similar variations occur in the shape and size of the pycnidia. All stages may appear, from a single large immersed pycnidium to a number of pycnidia almost free on the surface of the stroma. They may be spherical, cuneiform, oval or elongated vertically, especially in those long narrow stromata in which the pycnidia are compressed laterally (Pl. X, fig. 3). It appears that the first pycnidium to be formed is a single large cavity in an immersed stroma. The different types may be regarded as stages in the development, the course of which can be followed in the diagrams (Text-fig. 5 C).

A more detailed examination of the structure of the fruit-body was carried out on sections of embedded material. There is no evidence of any distinct pycnidial wall, *i.e.* the stroma is Dothideaceous.

The fruit-body, therefore, is a unilocular or multilocular stroma, the pycnidia being locules of variable size and shape and occurring at different levels in the stroma. They eventually appear almost free on the surface of an erumpent stroma. As in the pycnidia produced in culture, the spores are borne on slightly flexuous sporophores 8–10 μ long; no paraphyses have been seen (Plate X, fig. 4).

The *Torula* stage in nature

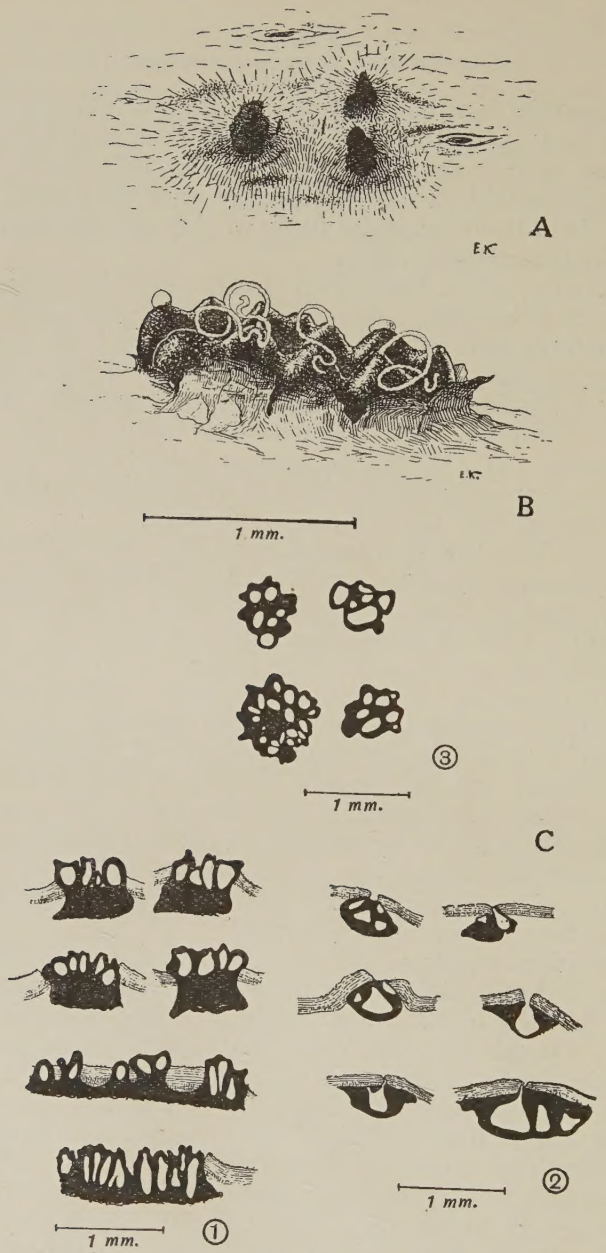
In host material that has been dead for a considerable time it is possible, in section, to trace old thick-walled hyphae in the wood vessels. These hyphae are frequently broken up into chains of thallospores and presumably would be liberated with the rotting away of the tissues. This, however, is not likely to play an important part in the dissemination of the fungus.

The occurrence of the *Torula* stage in a free state in nature was not observed until November, 1930, when a number of fructifications of an unusual form were seen. They were collected from material of a plum tree at Giza and did not resemble any of those previously examined. Such fructifications consisted of a number of slightly raised oval "blisters," with a longitudinal crack in the overlying tissue (Text-fig. 5 A). Within the cavities below were found masses of black powdery thallospores which in morphological and cultural characters agreed with the original *Torula* form.

Sections of these fructifications showed that the thallospores are borne on an immersed stroma or stromatic plate at the bottom of the cavity. In some of these developing stromata immature pycnidia were present. Scarcely discernible, hitherto unbroken, "blisters" were dissected out and were found to contain a flat circular or oval stromatic plate and an abundance of the *Torula* thallospores. Typical stromatic plates measured 0·5–0·6 mm. in diameter; the oval ones measured up to 1 mm. in length and 0·5 mm. in diameter.

From the many variable forms of stromata examined, it is possible tentatively to sketch the probable sequence of their development. As will be remembered from the account of the first observation of the pycnidial stage in nature, this is not necessarily preceded by the *Torula* stage. The Dothideaceous stromata are the fructifications which have been most usually observed.

The conditions under which the *Torula* stage appears, in conjunction with the pycnidial stage, have not been determined, but it is suggested that the fructifications begin as a flat stromatic plate, in the cortex. On this plate the *Torula* thallospores are formed in considerable numbers, developing into a compact mass, which produces the blister-like swelling of the tissue above. The developing stroma, meanwhile, causes the overlying tissue to burst, thus liberating the *Torula* spores. The stroma, at first, probably contains only a single large pycnidium and is completely immersed in the substratum. This phase corresponds to the single large pycnidia which are formed in the wood cultures. With further development daughter pycnidia appear in the upper portions of the stroma, while the original central locule becomes filled up with stromatic tissue. The final stage is the



Text-fig. 5. A, surface view of "blisters" enclosing *Torula* stage visible through fissure (× 28). B, surface view of erumpent stroma with protruding locules (× 28). C, transverse and horizontal sections of stromata showing different dispositions of the locules (× 28).

formation of almost free pycnidia on the surface of an erumpent stroma.

The fungus is widely distributed in Upper and Lower Egypt but has apparently not been recorded from other parts of the world, though a *Torula* having a close resemblance has been isolated from a "die-back" of apple in Cyprus.

In its general life history it presents a resemblance to such a fungus as *Coniothecium chromatosporum* Cda. from which, however, it differs in the partial blackening of the pycnospores. As the mature "fruit bodies" are here interpreted as multilocular stromata, the fungus is proposed as a new species of the genus *Hendersonula* with the following diagnosis:

Hendersonula toruloidea n.sp.

Stromatibus solitariis, immersis vel erumpentibus, uni- vel multi-locularibus, nigris, irregularibus, saepe elongatis, 1·5 × 0·5 mm. attingentibus. Loculis immersis vel protuberantibus, globosis v. e latere compressis v. irregularibus. Sporophoris hyalinis, teretibus, totos loculos vestientibus, 0-septatis, 8–10μ longis. Pycnosporis oblongis vel ovalibus, primo hyalinis, 0-septatis: dein saepe 2-septatis, fusco centrali cellulo praeditis, 12–13·5 (17) × 4–5·5μ. Hyphis primo hyalinis, septatis, ramosis, in moniliformes, globosas (4μ diam.) vel oblongas (10 × 4μ), vel cuneatas, fuscas 0- vel rariter 1-septatas thallosporas, quae ad *Torulam* vergent, secedentibus.

Summary

1. An account is given of a hitherto undescribed species of *Hendersonula* associated with a "die-back" of deciduous trees in Egypt.

2. The fungus possesses a *Torula* stage.

3. The *Torula* stage is linked up with a compound Sphaeropsid which shows a delayed browning and septation of the pycnospores after extrusion from the pycnidium.

4. The name *Hendersonula toruloidea* is proposed for the sphaeropsid form, a diagnosis of which is given.

Acknowledgments

I am greatly indebted to Mr E. W. Mason of the Imperial Mycological Institute for active help and interest throughout; particularly for invaluable help in preparing the paper.

Acknowledgments and thanks are due to Mr G. H. Jones, Chief Mycologist, for facilities for obtaining material for this paper while holding an appointment in the Ministry of Agriculture, Cairo.

EXPLANATION OF PLATES IX AND X

Plate IX

Fig. 1. Five isolations from diseased tissue, shown in reverse.
Fig. 2. Surface view of a three-day growth on prune-extract agar before darkening.
Fig. 3. Transverse section of two locules produced in culture on sterilised branch. × 180.
Fig. 4. Single stroma, erumpent on branch of plum tree. × 10.

Plate X

Fig. 1. Section of stroma with single locule still beneath periderm.
Fig. 2. Section of stroma showing rudiments of erumpent locules.
Fig. 3. Section of old stroma showing locules immersed and erumpent, globose, compressed and irregular.
Fig. 4. Section of lining layer of locule showing sporophores and hyaline pycnospores.

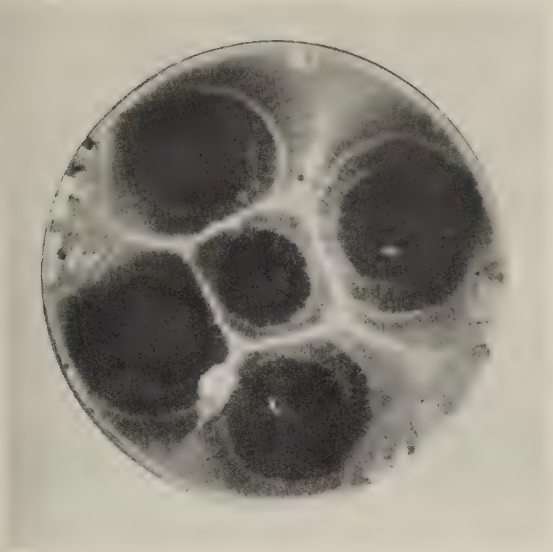


Fig. 1

Fig. 2

Fig. 3

Fig. 4

HENDERSONULA TORULOIDEA

AZYGOZYGUM CHLAMYDOSPORUM NOV. GEN. ET SP. A PHYCOMYCETE ASSOCIATED WITH A DISEASED CONDITION OF *ANTIRRHINUM MAJUS*

By CHAS. G. C. CHESTERS
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(With Plate XI and 4 Text-figures)

Introduction

The Phycomycete which is the subject of this paper was obtained in culture during attempts to isolate the organism causing a collar-rot and foot-rot of *Antirrhinum majus* (Pl. XI, fig. 1). The development of the perfect spore is in many respects different from that described for other Phycomycetes, and it has been impossible to place the fungus in direct relation to any described group. The early stages, which are initiated by the fusion of two hyphal branches, suggest an affinity with the Zygomycetes, but the later stages diverge from those so far described for fungi belonging to that group. The absence of a sporangial or conidial stage and the continual reproduction by means of chlamydospores render it difficult to assign a definite position to this fungus. Growth on a variety of media and under diverse conditions has failed so far to induce the formation of any other spore form. The perfect spore appears to be non-functional under cultural conditions and degeneration of its contents often occurs.

The fungus was present in the cortex of diseased plants and was associated with the actual pathogen which in some cases was *Rhizoctonia Solani* Kühn and in others *Phyllosticta Antirrhini* Syd. The seedlings affected were growing in cold frames. On the surface of diseased stems and especially on the roots numerous intercalary chlamydospores ornamented with short blunt spines were produced. These were also found in diseased soil in contact with the seedlings. They were spherical and measured from 10 to 26μ in diameter (Pl. XI, fig. 2). In one specimen large hyaline thick-walled spores about twice the size of the chlamydospores were observed (Pl. XI, fig. 2, bottom right-hand corner). Both spore forms have been developed in culture.

These chlamydospores were isolated and germinated on malt agar. The mycelium produced gave rise to chlamydospores of similar shape and size within a few days. On this medium and on several others the second spore form was later developed on single chlamydospore cultures. This spore was usually a bicellular structure consisting of

a thick-walled, hyaline or yellowish upper cell and a thinner-walled tapering stalk attaching it to the mycelium (Pl. XI, fig. 4 D). The thick-walled cell contained a single large oil drop occupying the major portion of the cell which was almost spherical and measured from 30 to 36μ in diameter. Such spores were also produced in sterilised garden soil after about fourteen days' growth.

Experimental methods

Of the several media used during this investigation the most satisfactory have proved to be Ashby's modification of Sherbakoff's maize agar(1), dilute malt-extract agar containing 2 per cent. of maltose sugar—determined by titration—and nutrient sucrose-nitrate agar containing 20 gm. sucrose, 2 gm. KNO_3, 0·5 gm. $MgSO_4$ $7H_2O$, 0·5 gm. KH_2PO_4, 30 gm. agar per litre of distilled water.

Thin films of mycelium which could be fixed and stained without disturbing the arrangement of the hyphae were obtained by placing sterile glass slides in plates of maize agar. Such preparations stained with cotton blue in lacto-phenol-glycerine (Amann's medium) were used to study the development of the two spore forms.

The development of individual thick-walled spores over a considerable period was followed by using a modification of a method suggested by Henrici(2), in which a cell of uniform depth was prepared by supporting a $2 \times \frac{7}{8}$ in. cover-glass on a microscope slide by means of three glass strips. These were cemented to the slide and cover-glass by beeswax and resin mixture. Such a cell, when filled with agar and inoculated, allowed observation of any particular portion of the mycelium and with ordinary care remained free from contamination for several days.

Material for cytological or detailed morphological study was fixed in Flemming's weak solution for one hour and then transferred to 1 per cent. chromic-acetic for twelve hours, washed in water for twenty-four hours and brought up to absolute alcohol through grades of 10 per cent. Microtome material was embedded in wax by the chloroform method or in collodion and then in wax. Sections were cut at 5 and 10μ and stained either in haematoxylin or cotton blue. The haematoxylin was prepared as a 0·5 per cent. solution in 50 per cent. alcohol and used after mordanting with a 0·25 per cent. solution of iron alum in 50 per cent. alcohol. A half-saturated solution of eosin in 50 per cent. alcohol was used as a counter-stain. At times half-strength Delafield's haematoxylin was employed and the sections were differentiated, after overstaining, in acid alcohol. The cotton-blue stain was prepared by dissolving 0·5 per cent. of cotton blue in Amann's medium (3). After staining for twenty-four hours the sections were differentiated in the lacto-phenol-glycerine mixture, washed in

95 per cent. alcohol, dehydrated, cleared and mounted in balsam. For general cytoplasmic structure this proved a useful and reliable stain, especially when examining hyphae embedded in plant tissue, since only the hyphae and their contents were stained.

The Phycomycete was isolated from several varieties of *Antirrhinum*, but only the strains from Beauty, Dazzler, Afterglow, Crimson Bedding and Appleby Matthews were retained for study. Isolations were made both from chlamydospores detached from the mycelium and from hyphae in infected tissue. Standard malt agar of *p*H 6·5 was used in making the isolations and for maintaining the stock cultures. Each stock culture originated from a single chlamydospore taken from an isolation plate.

Life history

(1) *The mycelium.* When chlamydospores are sown on malt agar and incubated at 24° C. each spore produces one or more long germ-tubes during the first eight to ten hours. These soon commence to branch and a small radial colony is established within twelve to fifteen hours. The hyphae at this stage are irregularly swollen and bead-like. These swellings are characteristic of the mycelium at all stages of its development (Text-fig. 4 A, H and J). On media containing nitrate or peptone they are very marked and each swelling is densely filled with oil drops. The ultimate branches of the immersed mycelium generally arise from such swellings and are very finely attenuated, especially on maize agar. Within the cortex of sterilised *Antirrhinum* stems the swellings are much exaggerated. Beginning as an irregularly enlarged branch (Text-fig. 4 B and C) numerous globular swellings are formed over its surface. From each of these a hypha is formed which may itself be tapered or may give rise to a series of attenuated branches (Text-fig. 4 D) which penetrate the cortical cells in all directions. These large masses of filaments have been observed only in culture.

The mature hyphae contain rather granular protoplasm in which small nuclei are embedded. At first the protoplasm is dense, but later it assumes a reticulate appearance due to the presence of numerous vacuoles (Text-fig. 4 A). The oldest hyphae are filled with oil drops and septa occur at intervals.

(2) *The chlamydospores.* From thirty-six to forty hours after germination has begun mature chlamydospores are present on the mycelium. These generally arise on small lateral branches but may occur on the larger branches. On small lateral branches only one chlamydospore is usually formed (Text-fig. 3 A, B and E). Although these are typical chlamydospores in the accepted meaning of the term they differ from those described for most Phycomycetes in that they do not appear

at a late stage in development when the hyphae are undergoing degeneration but are formed early in the life of the mycelium. They have a double wall and may be covered with short blunt spines which are outgrowths of the outer wall (Text-figs. 1 A and 3 E). When the chlamydospores are produced in moist air or in water the spines are longer and sharper than those produced on superficial or immersed spores. They are perfectly spherical and measure from 10 to 26μ in diameter. They have numerous small nuclei (Text-fig. 3 C–E) and the density of their protoplasm gives them a dark appearance when young, but they are not pigmented and, as they age, oil drops accumulate in their protoplasm. Their production continues throughout the life of the mycelium. The immersed forms are ovoid or slightly irregular and may often have no spines and a comparatively thick wall (Text-fig. 1 C and D). In old cultures portions of the hyphae are simply divided off into rather thick-walled segments (Text-fig. 1 E and F). These are more akin to the chlamydospores of other Phycomycetes.

(3) *The thick-walled spores.* While the chlamydospores appear soon after germination the other spore form does not reach its adult state until some five to fifteen days have elapsed. These spores occur either on the surface or immersed hyphae.

Maize and sucrose-nitrate agars were employed to study the details of the development of the second spore form. This spore is initiated at about the same time on all media but earliest on sucrose-nitrate agar, due, no doubt, to a certain lack of available food materials which gives rise to a sparse vegetative growth, and latest on malt agar on which abundant vegetative growth occurs. The spore is always initiated by the fusion of two hyphae (Text-fig. 2 A–C). These are usually two small lateral branches (Text-fig. 2 A and C), but often the end of a small branch fuses with an undifferentiated portion of another branch. In either case no active growing together of the fusion hyphae has been observed. On the contrary it would appear that in the first instance the branches must be touching. The two branches may arise from the same (Text-fig. 2 A) or from different parent branches (Text-fig. 2 B–F). Even in the youngest condition the two hyphae may be distinguished from one another in size. The larger is destined to become the stalk of the thick-walled spore. Independent of the type of medium on which they develop, a difference of structure becomes evident about the time when the branches, having fused, begin to enlarge. The smaller branch is always more hyaline than the larger (Text-fig. 2 D–F), and its protoplasm appears reticulate in structure. At this time the rudiment is distinctive, consisting of the two swollen ends of the attached hyphae and their thinner basal portions. These basal parts may branch to a varying degree. On maize agar very numerous tapering branches are formed,

some being of considerable length, on malt fewer and shorter branches occur, while on sucrose-nitrate agar there are practically no branches at all. As the two hyphae increase in size the smaller loses its hyaline and reticulate structure and becomes more or less granular (Text-fig. 2 E). It increases in size more rapidly than the larger and often the contents of the stalk become depleted in the process. When the two terminal portions are about the same size a septum is formed cutting off the originally smaller portion from its stalk (Pl. XI, fig. 4 A), which then degenerates entirely except sometimes for a minute tail above the septum. The stalk of the other branch remains attached and becomes thicker towards the swollen upper part. The walls of both parts thicken, oil appears in the protoplasm and the whole contents become dense and somewhat brown. Subsequent development may take place in either of two ways. Either the wall separating the two attached branches may become absorbed in part or completely, or the wall may remain and that of the terminal cell increase in thickness.

The first type of development occurs relatively rarely. It is a condition characteristic of a certain degree of maturation. Dissolution of the dividing wall only takes place after the deposition of secondary wall material has begun in either or both attached hyphae. This wall may break down towards the centre (Pl. XI, fig. 3 A) and later throughout its length, or several pores may appear (Pl. XI, fig. 3 B) and islands of the wall become separated. A thick wall is formed around the combined protoplasts, in which oil has begun to accumulate. The spherical or pear-shaped fusion cell is still attached to the mycelium by a single well-defined stalk.

Such large fusion cells have been kept under observation for several months, and a few have been isolated from the mycelium, but under various experimental conditions no germination has been observed. While attached to the mycelium they either do not change in appearance—except for a slight yellowing of the wall—or the wall becomes greatly thickened and partially closes the pore between the two parts (Pl. XI, fig. 3 C and D). In the latter condition oil rapidly accumulates and the protoplasm becomes disorganised.

The second course of development is the more usual. The contents of the terminal cell aggregate towards its centre forming a dense zone surrounded by a lighter area in which oil drops appear. Later the central mass becomes filled with oil drops (Pl. XI, fig. 4 B) which increase in size and coalesce (Pl. XI, fig. 4 C) until a single large globule is formed enclosed by an almost clear peripheral zone (Pl. XI, fig. 4 D). During this time the wall has increased in thickness. The basal cell, attached to the mycelium, retains its granular structure for a longer period (Pl. XI, fig. 4 D), but in time it also undergoes a similar series of changes. Often the whole contents of this cell degenerate and the terminal cell is set free.

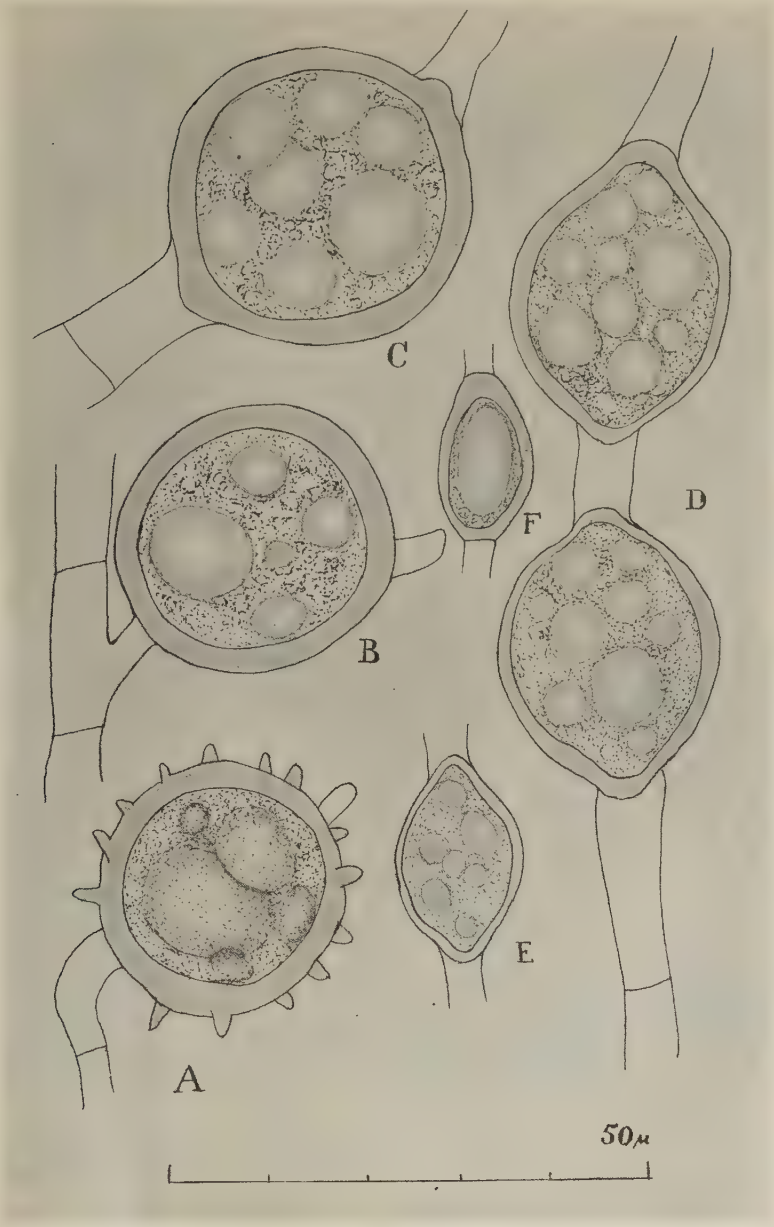
The cytological examination of such stages in the development of the pairs of attached hyphae, as have been described above, merely confirms the fact that any communication which is established between the two elements is formed at a comparatively late stage.

The earliest stages examined exhibit that differentiation in the size and character of the cell contents of the two hyphae which has already been noted (Text-fig. 3 F–H), and in progressively older stages the gradual increase in the size of these parts is observed (Text-fig. 3 J) until both elements are of approximately equal dimensions (Text-fig. 3 K). Up to this point the walls of the two hyphae have remained thin, and numerous small nuclei are fairly distinct. Very little oil is present in the two structures. Further increase in size takes place, oil appears in the contents of the cells and the walls begin to thicken (Text-fig. 3 L) in such a manner that a three-layered wall is formed. Each layer gives a distinct staining reaction. The outer is yellowish and does not stain easily or permanently; the middle layer, which varies in breadth, stains densely with haematoxylin; and the inner, comparatively thick layer, stains pale pink or salmon colour with eosin or congo red. This latter layer may increase in thickness during later stages of development.

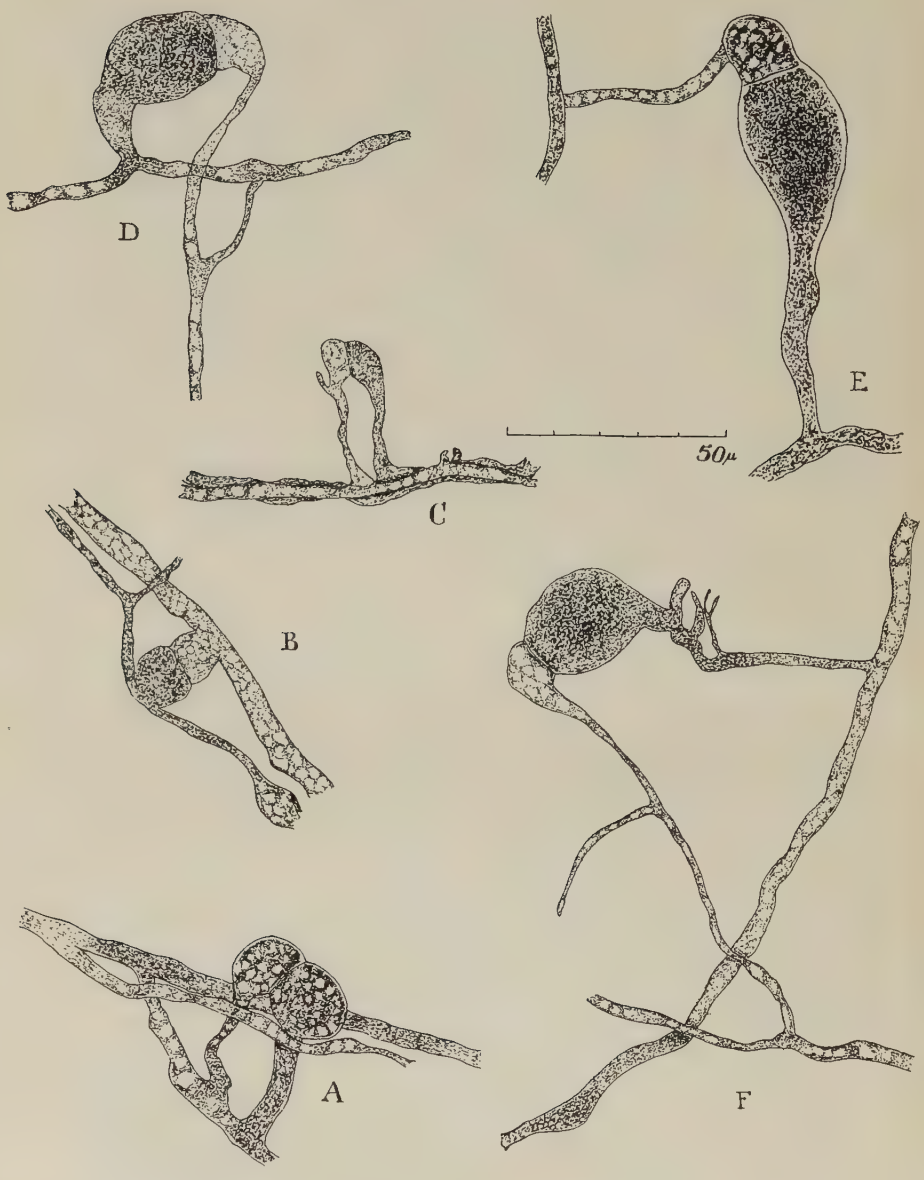
Where open communication is established between the two attached cells the breakdown of the dividing wall is initiated by changes occurring in the central layer which begins to swell up at one or more places throughout its length (Text-fig. 3 L). These swellings increase in size and one or more small pores appear in the wall (Text-fig. 3 M). When only a single pore is formed this becomes enlarged, but when more than one occurs islands of wall material are separated and lie embedded in the protoplasm of the cell or cling to the wall (Text-fig. 3 O). The large fusion cell (Text-fig. 3 P) has numerous nuclei and a prominent and fairly thick inner wall which extends throughout the cell. The remains of the dividing wall are still visible (Text-fig. 3 P).

Where communication fails to be established between the two cells there is no visible swelling of the middle layer of the wall (Text-fig. 3 N), but a small pore may penetrate the inner wall. The contents of the two cells become replaced by oil which ultimately occupies the centre of each cell and is surrounded by a mass of degenerate protoplasm. The basal cell may lose its contents. The majority of the spores examined show that abnormal development of the innermost layer of the wall initiates the degenerative processes.

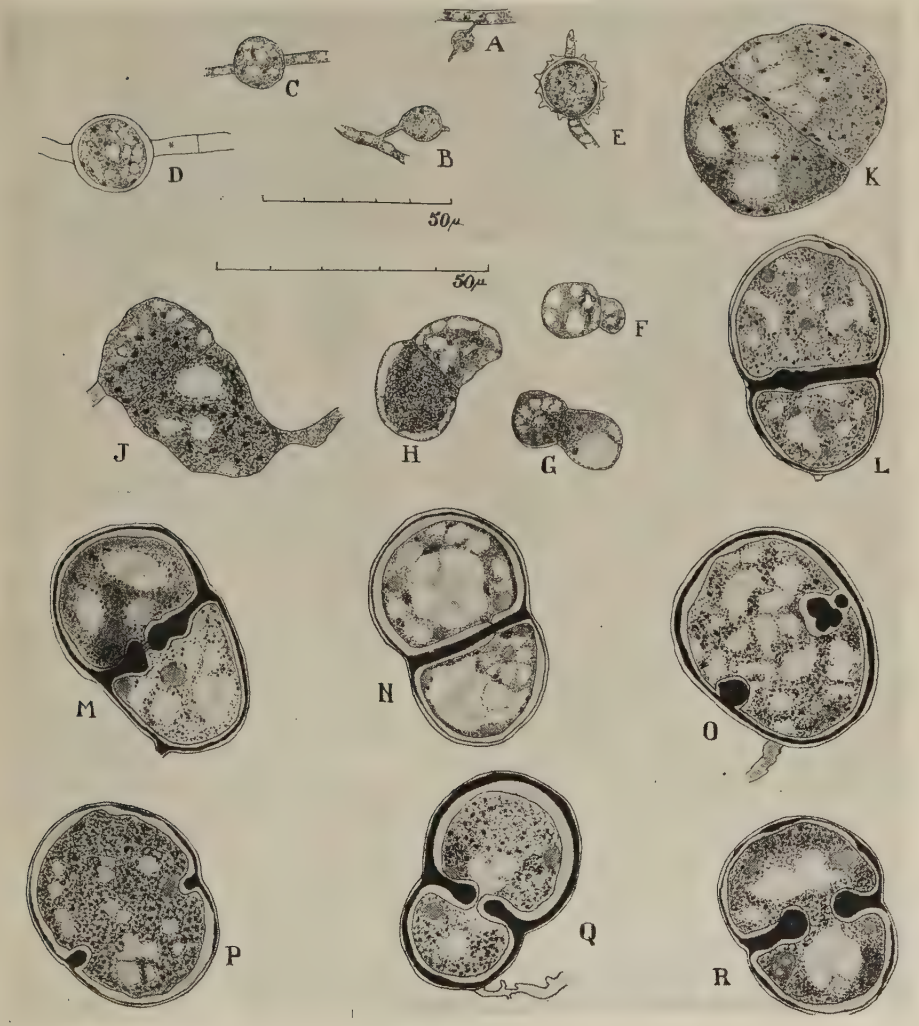
A proportion of those in which a fusion cell is formed appear to degenerate. This condition exists when the pore is established after much thickening of the inner cell layer has occurred and when a very localised swelling of the middle layer takes place (Text-fig. 3 Q and R). Here the contents quickly degenerate and are replaced by oil, and



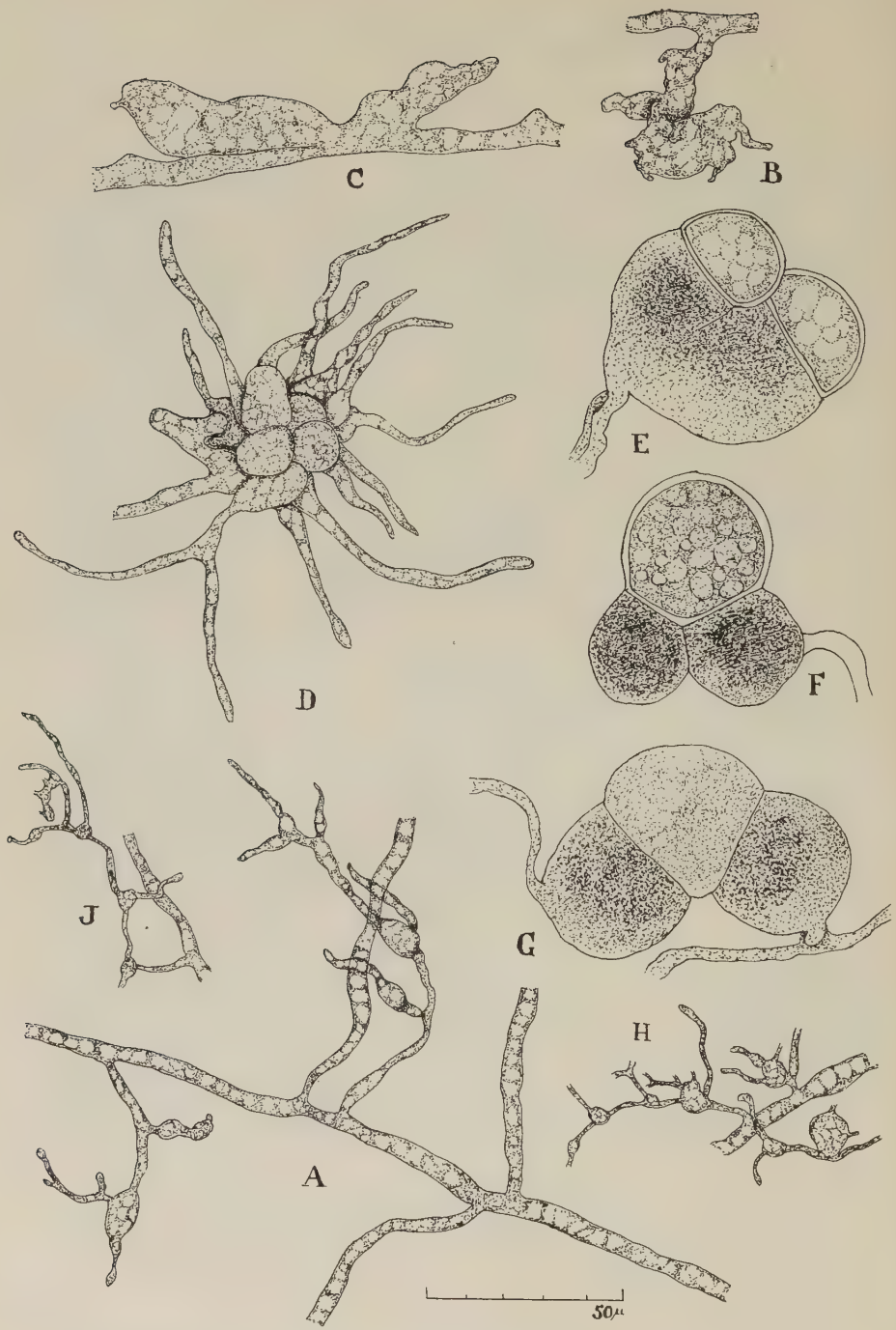
Text-fig. 1. Chlamydospores. A, from aerial mycelium on malt agar; B, from immersed mycelium; C–F, from deeply immersed parts of the mycelium of an old culture on malt agar.



Text-fig. 2. Young fusion hyphae. A–E, from sucrose-nitrate agar; F, from maize agar.



Text-fig. 3. Sections of various stages in the development of the chlamydospore and the thick-walled spore. A–E, development of the chlamydospore; F–K, early stages in the development of the thick-walled spore; L–R, later stages of the same.



Text-fig. 4. Appearance of the mycelium. A, H and J, in malt agar; B, C and D, in *Antirrhinum* stems; E–G, certain fusion hyphae (for further explanation see text).

the inner wall thickens to such an extent as almost to close the pore (cf. Pl. XI, fig. 3 C and D).

(4) *Cultural characteristics.* The growth characteristics of the fungus are so clearly defined on all media that a summary of them seems fitting. The chief results of the cultural treatments given below are summarised in the table.

Media used	Aerial mycelium	Surface mycelium	Submerged mycelium	Chlamydo-spores	Resting spores	Comparative growth
(1)	White dense zoned	Thick zoned	Numerous groups round inoculum	Aerial surface submerged	Surface and submerged	Dense, zoned growth
(2)	White fluffy	Fairly thick	Groups spreading from inoculum	Aerial surface submerged	Mainly submerged	Good growth
(3)	None	Thin	A little but irregular	Surface	None	Sparse growth
(4)	White fairly dense	Very thick	Thin over all cultures	Aerial	None	Dense, zoned; liquifies gelatine in 10–14 days
(5)	Hardly any	Thin spreading	If present—as groups	Aerial surface submerged	Mainly submerged	Fair growth
(6)	Small amount	Fairly thick	A few groups	Aerial surface submerged	None	Good growth, slightly staling
(7)	White fluffy	Thick	Thick over all culture	Aerial surface	None	Dense growth, staling
(8)	Very thin	Thin spreading	Dense and deep	Aerial surface submerged	Submerged	Mostly embedded in the tissues
(9)	Hardly any	Thick	General	A few on surface	None	Fair
(10)	Hardly any	Thick	General	A few on surface	None	About twice as much as on (9)

Media used

(1) Malt extract containing 2 per cent. maltose, 3 per cent. agar.
(2) Maize agar (Ashby's formula(1)).
(3) Potato agar (Brown's formula(4)).
(4) Malt extract gelatine, 12 per cent. gelatine.
(5) Synthetic medium (see p. 200) + 0·2 per cent. KNO_3.
(6) Synthetic medium (see p. 200) + 0·2 per cent. peptone.
(7) Synthetic medium (see p. 200) + 0·4 per cent. peptone.
(8) Steam sterilised *Antirrhinum* stems.
(9) Synthetic medium + 2 per cent. (vol.) olive oil.
(10) Synthetic medium + 2 per cent. (vol.) castor oil.

On any synthetic medium the greatest growth is obtained with maltose as the source of carbohydrate and KNO_3 as the source of nitrogen. Substituting glucose for maltose the amount of growth is almost the same but less aerial mycelium is formed. Sucrose is a

poorer source of carbohydrate. When an organic source of nitrogen is substituted for KNO_3 staling and abnormal development of the mycelium occur in proportion to the concentration of the substance used.

The fungus grows abundantly as a saprophyte in ordinary garden soil, producing both spore forms. When *Antirrhinum* seed is sown in such infected soil the seedlings produced remain comparatively healthy and no clear case of parasitism by the fungus has so far been observed.

Discussion

There is no doubt that this fungus must be placed in the Phycomycetes. The eseptate mycelium consisting of rather coarse hyphae filled with granular protoplasm and numerous small nuclei is characteristic. This mycelium bears both chlamydospores and large thick-walled spores which arise by the fusion of two differentiated hyphal branches. Its exact position will be determined by the interpretation placed on the character of the mycelium, the thick-walled spores and the chlamydospores.

(1) *The mycelium.* The mycelium is extensive whether it occurs on diseased *Antirrhinum* roots, in infected soil or in culture on various media. This indicates a relationship with the higher rather than with the lower Phycomycetes. Within the Chytridiales, as they are delimited by Fitzpatrick (5), the mycelium, if present, is poorly developed. Recently a new genus of the Cladochytriaceae has been described by Sideris (6) under the name *Rhizidiocystis*. The only species, *R. Ananasi*, has an extensive mycelium parasitic on pineapple roots. In general appearance it bears a similarity to the fungus on *Antirrhinum*. Both develop on roots and produce a fluffy white mycelium. The hypnospores of *Rhizidiocystis* and the chlamydospores of this fungus are of somewhat similar shape and size. The former fungus produces zoosporangia. Such structures have never been observed during the course of the present investigation. *Rhizidiocystis* has an evanescent mycelium, while this has a persistent mycelium. Any similarity is superficial, and there is no doubt that this fungus should be placed in a higher order.

(2) *The thick-walled spore.* The absence of a motile stage in any part of the life history suggests a position near to the Zygomycetes, and this is supported by the development of the thick-walled spore. The value to be placed on these developmental stages can be assessed only after the following features have been considered. The fundaments of the thick-walled spore are two attached, disproportionate hyphae. The originally smaller hypha becomes abstricted from the mycelium after a period of growth. Communication between the two parts may never be established, and the whole structure may become bicellular

with a thick-walled distal cell. Communication may occur and a thick-walled fusion cell be formed. The formation of a fusion cell is a late phenomenon.

In all described Zygomycetes the hyphae which fuse to give the zygospore are recognised as progametangia, each of which later becomes differentiated into a basal persistent suspensor and a terminal gametangium. Only the two gametangia take part in the production of the zygospore whose thickened wall is laid down within that of the fused gametangia. At maturity the zygospore remains attached to both suspensors.

This Phycomycete shows no differentiation of the attached hyphal branches unless it be considered that the tapering basal parts of the two branches represent suspensors, and, even if this be so, one of these is not persistent. Although the earliest stages in the formation of the thick-walled spore are reminiscent of zygospore production subsequent development proceeds along different lines, and either a bicellular structure, in which the upper portion becomes spore-like, or a large fusion cell, attached basally, is formed. The former condition has no parallel in the Zygomycetes. Azygospores are known in several genera under certain cultural conditions, but these always arise from one gametangium and not, as here, from an isolated part of one hypha attached to another. The latter condition is also atypical. It occurs after thickening of the cell wall of the two elements has taken place and not, as in the zygospore, while the fused hyphae are still young. Also, the wall of a zygospore is a new structure formed around the fused protoplasts, while here the thick wall is merely an extension of a structure already present.

The occurrence of spores in which an extremely small pore is formed or in which increase in the depth of the inner wall almost occludes the pore indicates that the whole process is extremely unstable. This is emphasised by a very few observations of young, apparently typical zygospores (Text-fig. 4 G) formed between swollen parts of two fused hyphae. These were produced on a sucrose-nitrate culture, but attempts to repeat their formation have so far failed. On malt agar others were observed (Text-fig. 4 E and F) in which either the basal or the distal parts of similar structures were fused together. These three examples are interpreted as indicating that the fusion elements seen in all cultures are really zygospore-like rudiments, but that for some reason development is abortive. The cause of this does not appear to lie in the fact that the fungus is heterothallic, as all isolations behave alike and when grown against one another fail to produce zygospores but do produce thick-walled spores.

I am of the opinion that the initial stages in the development of the second spore form of this fungus can be legitimately compared with

the initial stages in zygospore production, and on that account place the fungus in the Zygomycetes. Later development is regarded as abortive, and the special instances illustrated in Text-fig. 4 are thought to indicate that, if correct conditions were established, normal zygospores would be produced.

(3) *The chlamydospores.* These structures are produced on almost every medium, and, if the fungus has been recorded previously, at least the chlamydospores should have been described. They are always intercalary, sometimes singly, sometimes in series. Spines, which are outgrowths of the outer wall, occur when the chlamydospores are aerial or are formed in water. Transitional stages exist between these aerial chlamydospores and others formed from thick-walled mycelial segments which occur immersed in the substrate.

A search through available literature has failed to discover any imperfect genus having chlamydospores of the type described. *Chaetoconidium* possesses chlamydospores which are somewhat similar, but they are produced on a septate mycelium (7). The genus *Humicola* was created by Traaen for two soil fungi having chlamydospores (8)*. There is general resemblance to *H. grisea* but there are marked differences. The chlamydospores of the latter are usually solitary, seldom in chains and never regularly intercalary. They are apparently not ornamented and the mycelium is brown or dark coloured.

Within the Zygomycetes several genera produce chlamydospores, and in *Mortierella* those on the aerial mycelium are specialised (stylospores). They resemble the aerial chlamydospores of this fungus in size and in the fact that they are ornamented, but they are generally produced singly upon short upright branches. The development of the stylospores of *M. polycephala* has been described by Dauphin ((9) p. 41 *et seq.*). The spore (*loc. cit.* Fig. 33) is remarkably similar to the chlamydospore of this species except that it is not an intercalary structure. Dixon-Stewart (10) has described a new species of *Mortierella* —*M. stylospora*—which was isolated from garden soil. She states that: "Reproduction in culture appears to be by means of well-developed stylospores." The latter are produced on upright aerial hyphae 40μ in length and 18μ in diameter. Rudimentary zygospore masses are also formed, but the zygospore does not mature in culture.

As far as the chlamydospores are concerned this Phycomycete is more closely related to *Mortierella* than to any other genus of the Zygomycetes, but the affinity, if it exists, cannot be stressed as zygospores of a type similar to *Mortierella* have not been observed. It must be realised also that the chlamydospores of a fungus are not always a reliable criterion of its relationship. The branching of the basal parts of the fusion hyphae (cf. p. 202 and Text-fig. 2 F) may not

* I am indebted to Mr Ramsbottom for bringing this genus to my notice and for supplying an abstract of the original description.

be without significance, as it is from the suspensors that the hyphae investing the zygospore of *Mortierella* are produced (9).

It is not intended to suggest that this Phycomycete is a species of *Mortierella*, but it is considered that its position in the Zygomycetes is certainly in close proximity to the Mortierellaceae.

Azygozygum nov.gen.

Mycelium effusum, persistens; hyphae eseptatae, ramis alternis, ramulis ultimis exquisite attenuatis.

Chlamydosporae intercalares, solitariae v. seriatae, sphericae v. ellipsoideae, cortici v. substrato immersae, episporio laevi instructae, superficiales spinis ornatae.

Sporae vulgo abortivae, bicellulares, cellula terminali laevi crassitunicata, basali tenuiter tunicata, inferne attenuata; at maturae zygosporicae, crassitunicatae, laeves, ferme sphaericae, mycelio basi adnatae.

Sporangia aut conidia nulla visa.

Habitat ad quisquilias putridas in horti solo jacentes.

A. chlamydosporum nov.sp.

Mycelium effusum, persistens, album; hyphae eseptatae, oleo copioso farctae, irregulariter tumefactae, ramis alternis, 3–5μ diam., ramulis ultimis attenuatis, ex hypharum tumoribus oriundis.

Chlamydosporae numerosissimae, intercalares, solitariae v. seriatae, superficiales v. immersae; superficiales achroae v. lutescentes, sphaericae, 10–26μ diam., spinis obtusis praeditae, in matrice immersae ellipsoideae, laeves, crassitunicatae, luteolae.

Sporae vulgo abortivae, bicellulares, cellula terminali crassitunicata, laevi, luteola, ferme sphaerica, 30–36μ diam., basali tenuitunicata, achroa, magnitudinis aequalis, inferne attenuata; at maturae zygosporicae, crassitunicatae, laeves, luteolae, fere sphaericae, 40–60μ diam., achroae v. lutescentes, mycelio basi adnatae.

Plerumque matrici immersum est. Ex *Antirrhini* stipitibus radicibusque, *Rhizoctonia Solani* Kühn affectis, aeque ac ex horti solo prope Birmingham Angliae excerpsi et arte colui.

Summary

1. The fungus was found associated with a diseased condition of *Antirrhinum majus* due to *Rhizoctonia Solani* and at times to *Phyllosticta Antirrhini*.

2. The appearance of the spiny chlamydospores attached to eseptate mycelium on the diseased plants and in the soil is described.

3. The organism has been grown in culture.

4. Chlamydospores similar to those on the host are developed in culture. Also a second spore form which may be either a bicellular

structure or a thick-walled fusion cell. These arise by the fusion of two hyphal branches.

5. Neither of the two thick-walled structures has been germinated.
6. Cultural characteristics are dealt with.
7. It has been found impossible, so far as experiments have gone, to induce this fungus to attack living *Antirrhinum* plants.

The writer wishes to acknowledge the kindness of Mr W. B. Grove in translating the diagnoses into Latin, and much helpful criticism received during the course of this investigation from Dr E. J. Butler, Mr S. F. Ashby, and Mr J. Ramsbottom.

EXPLANATION OF PLATE XI

Fig. 1. *Antirrhinum* plants suffering from collar-rot from which the Phycomycete was obtained.
Fig. 2. Chlamydospores and two thick-walled spores in the cortex of diseased *Antirrhinum* stems.
Figs. 3, 4. Stages in the development of the fusion hyphae.

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Fig. 1

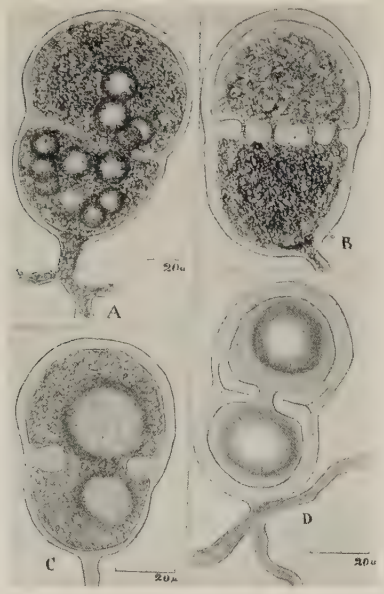

Fig. 3

Fig. 2

Fig. 4

AZYGOZYGUM CHLAMYDOSPORUM

NEW CHYTRIDIACEOUS FUNGI

By F. K. SPARROW, Jr.

In the course of my studies of fresh-water fungous life pursued during the past year (1932–3) at the Botany School, Cambridge, of the numerous Phycomycetous fungi observed, several Chytridiaceous organisms were encountered which were seemingly new to science.

It has seemed desirable for purposes of record to characterise briefly at this time these new forms, leaving for a later paper a more detailed and illustrated account of them.

I am greatly indebted to E. F. Warburg, Trinity College, Cambridge, for the material which he brought back from north Africa, and also for his kindness in translating into Latin diagnoses of the new forms found.

The following new fungi are recorded:

Septolpidium gen. nov. Thallus intramatricalis, cylindricus, irramosus, maturitate transverse divisus in seriem segmentorum conjunctorum, quidque maturitate processum evacuantem unum formans et per eum zoosporae exonerantur. Zoosporae posteriore uniciliatae.

S. lineare nov.sp. Thallus anguste cylindricus, irramosus, finibus leviter deminuentibus, 75–130μ longus, 5–12μ diametro, maturitate transverse divisus in seriem segmentorum cylindricorum levium truncatorum conjunctorum, 8–37μ longorum, quidque maturitate processum evacuantem unum, latum, abrupte deminuentem, circa 3–5μ longum, 3–5μ diametro formans qui e cellula matricis protruditur et per quem zoosporae exonerantur. Zoosporae sphaericae, posteriore uniciliatae, 3–4μ diametro, cum 1–4 granulis refringentibus, formae inaequalibus; plerumque, cum emergunt e sporangio, capitulum immobile, nonnihil sphaericum, ad ostium processi evacuationis qui degenerat cum zoosporae movent, formantes. Sporae perdurantes non observatae.

In *Synedra* sp., Chapman's Garden, Emmanuel College, Cambridge; September, 1932.

Pleolpidium Polyphagi nov.sp. Sporangia sphaerica pellucida in prosporangiis matricis saepe multum turgidis, formata; 20–48μ diametro; maturitate cum 2–6 papillis 4–8μ diametro per quem zoosporae numerosae, minutae, posteriore uniciliatae, uniguttulatae, anguste ovoideae, 2–3μ longae, 1·5–2μ diametro, exonerantur. Sporae perdurantes non observatae.

In prosporangia of *Polyphagus Euglenae*, ditch, rear of Leys School, Cambridge; March, 1933.

Rhizidiopsis nov.gen. Zoospora, cum restiterit, cystum formans et rhizoides intramatricales pariens; processum germinandum extramatricalem, latum formans qui se amplit et sporangium fit. Zoosporae posteriore uniciliatae, intra sporangium plene formatae, postquam papilla delicuit, exonerantur. Sporae perdurantes extramatricales, membranis crassis, sporangium simile illo zoosporae parientes.

*R. emmanuelensis** nov.sp. Sporangium, membrana levi, piriforme, 6–12 μ longum, 5–10 μ diametro, axe longo parallelo filo algae, per finem angustiorem cysto rotundato zoosporae, 3–4 μ diametro, annexo. Sporangium 4–12 zoosporas, 3–4 μ diametro formans, quae maturitate per foramen unum apicale postquam papilla delicuit, exonerantur. Sporae perdurantes sphaericae, membranis crassis, 4–4·5 μ diametro, fusce et aspere incrustatae.

Parasitic on *Melosira varians*, *Nitzschia* sp., Chapman's Garden, Emmanuel College, Cambridge; March, 1933.

Podochytrium lanceolatum nov.sp. Sporangium pellucidum, lanceolatum, membrana levi, 8–10 μ diametro, finibus deminuentibus 3–4 μ; super basi sterili, cupulae simili, 4–5 μ diametro, restans; cum processu intramatricali uno, acui simili, ex apice cujus rhizoides pauci emanant. Zoosporae sphaericae, 3–4 μ diametro, uniguttulatae, posteriore uniciliatae, ex sporangio extrusae postquam papilla parva apicalis delicuit. Sporae perdurantes non observatae.

Parasitic on *Melosira varians*, Chapman's Garden, Emmanuel College, Cambridge; December, 1932.

Scherffelia† nov.gen. Zoospora in aqua libra jacens, cum germinat, processum angustum pariens, cujus apex ad cellulam matricis per mycelium intramatricale annectitur; apex extramatricalis processus germinandi expandit et sporangium fit ad quod cystus vacuus zoosporae annectitur. Zoosporae posteriore uniciliatae, postquam una papilla delicuit ex sporangio exonerantur. Sporae perdurantes extramatricales, membranis crassis, simulate similiter sporangio formatae.

S. parasitans nov.sp. Sporangium primo lacrimosum, postea sphaericum vel subsphaericum, in concavitate per cellulam collapsam matricis formata, restans; 17–22 μ diametro, per processum angustum ad cystosporam sphaericam, 2·5–3 μ diametro junctum; cum processu intramatricali, vix visibili, uno, brevi, irramoso; pars interior primo pellucida, maturitate cum guttulis aurantiacis olei instructa. Zoosporae minutae, numerosae, sphaericae vel ovoideae, 2–3 μ diametro, quidque cum guttula una aurantiaca olei; in capitulo ex ostio lato

* So named not only to recall a site exceedingly rich in all types of aquatic fungi, but also to express my thanks in an all too inadequate manner to the Master and Fellows of Emmanuel College, Cambridge, for their many kindnesses during the progress of the investigation.

† Named after Dr Aladar Scherffel, the distinguished Hungarian student of the Chytridiales.

sporangii emergentes et per tempus brevissimum ad ostium manentes; capitulum tandem discussum cum zoosporae movent. Sporae perdurantes sphaericae vel ovoideae, membranis crassis subfuscis, 8–10μ long. v. 10–14μ diametro per processum angustum, saepe satis curvatum, ad cystosporam unam, 3μ diametro annexae.

Parasitic on the resting cysts of *Euglena* sp., ditch, Grantchester Road, Cambridge; March, 1933.

Sporophlyctidium nov.gen. Zoospora in aqua libra jacens, cum germinat, processum unum irramosum, satis inflatum, rectum, longitudine variante, cujus apex cellulam matricis penetrat, parit; corpus zoosporae expandit et sporangium fit. Zoosporae maturitate ex sporangio per papillam deliquescentem liberatae; forsan sine ciliis.

S. africanum nov.sp. Sporangium anguste ovoideum vel piriforme, membrana levi, pellucidum, fine angustiore cum processu germinando irramoso continente; 5μ longum, 3·5–4μ diametro; foramen unum subapicale per quod sporae sphaericae uniguttulatae, 2μ diametro extruduntur, formans; cilia et motus zoosporarum non observata.

Parasitic on *Protoderma* sp., which appeared in water cultures of vegetable matter collected by E. F. Warburg in Tangier in April, 1933.

This work was done while the writer was the holder of a National Research Council Fellowship in the Biological Sciences.

TWO NEW RECORDS OF PHYSIOLOGIC FORMS OF WHEAT-STEM RUST IN KENYA COLONY

By J. McDONALD, D.F.C., B.Sc., F.L.S.

In a previous paper(1), a description was given of investigations carried out in Kenya Colony which led to the discovery of the existence in that country of two distinct physiologic forms of wheat-stem rust (*Puccinia graminis tritici* Eriks. & Henn.). These forms, which are known locally as K 1 and K 2, were used to inoculate seedlings of the range of wheat varieties adopted by Stakman and Levine in America(2) as differential hosts for the identification of physiologic forms of stem rust. As a result, forms K 1 and K 2 were found to produce reactions on these varieties corresponding with those recorded by Stakman and Levine for American forms 21 and 17 respectively.

The purpose of the present paper is to place on record the existence of two further physiologic forms of stem rust which have since been found in the Colony. One of these forms was also identified in a rust collection received from the neighbouring country, Tanganyika Territory.

The methods used in these investigations were identical with those described in (1), p. 237, and the reactions of the various differential hosts to infection were recorded, as before, twenty-one days after inoculation and according to the scale of values devised by Stakman and Levine(2).

In September, 1930, a sample of stem rust from a field of Reliance wheat growing at Njoro (7100 ft.) was received from Mr R. J. Lathbury (Plant Breeder) for determination of the physiologic form present. The usual inoculation tests on seedlings of the American differential hosts were made and, as a result, this Njoro sample was found to differ from the forms previously discovered to exist in Kenya in its ability to produce a highly susceptible type of reaction on both Kanred and Reliance wheats. This, of course, was to be expected with Reliance wheat, as the field collection was made from that variety. The similar reaction on Kanred, however, provided interesting evidence in support of American experience that Reliance wheat may be substituted for the Kanred variety as one of the differential hosts, as they always react similarly to each other with whatever form of stem rust they are inoculated.

Many subsequent tests have confirmed the separate identity of this physiologic form. It has been designated K 3 and its full list of reactions on the American differential hosts is given below (Table I),

together, for comparison, with those of the other forms known to occur in Kenya.

The presence of a fourth physiologic form was established as the result of tests made with a collection submitted by Mr G. J. L. Burton (Senior Plant Breeder) of stem rust which he observed in a field of Kenya Standard wheat at Ulu (4500 ft.), about fifty miles south-east of Nairobi. This wheat, which had been bred by Mr Burton, had proved highly resistant to the three previously known forms.

The feature in which the Ulu rust sample, when tested on the American differential hosts, differed from all previous collections made in Kenya was the susceptible reaction induced on Vernal wheat inoculated with it. The full list of reactions of this physiologic form, which is known locally as K 4, is given in Table I, in which, following Stakman and Levine(2), infection types 0, 1 and 2 are classed as resistant and types 3 and 4 as susceptible.

Table I. *Reactions produced on American differential hosts by the four physiologic forms so far known to exist in Kenya*

(R=resistant, S=susceptible)

Variety	Physiologic form K 1	K 2	K 3	K 4
Einkorn	R	S	R	R
Vernal	R	R	R	S
*Kanred	R	R	S	—
*Reliance	R	R	S	R
Khapli	R	R	R	R
Kota, Acme, Arnautka, Mindum, Kubanka, Marquis, Spelmar, Little Club	S	S	S	S

* Interchangeable.

Identification of Kenya physiologic forms with those described elsewhere

It is a matter of interest to try to identify physiologic forms occurring in Kenya with those described from other countries. As already pointed out in (1), forms K 1 and K 2 appear to be identical with American forms 21 and 17 respectively.

Of the forms now recorded, K 3 appears to show a clear-cut agreement with the reactions of American form 34. Form K 4, however, calls for more cautious treatment.

Many workers have drawn attention to the variability in reaction to inoculation which can be brought about by changing experimental conditions. At Nairobi, where the present investigations were carried

out, it has not been possible to modify greatly the conditions imposed by the natural climatic factors. Thus, at certain periods of the year maximum day temperatures remain below 70° F. for days together, and there are long periods of overcast weather with low light intensity. At such times infection values are noticeably depressed compared with those obtained during the months of bright sunshine when maximum temperatures are from 10 to 15° F. higher. This has, however, occasioned little difficulty in deciding infection types for forms K 1, K 2 and K 3 on the American differential hosts but, with form K 4 one or two varieties, in particular Marquis and Arnautka, have sometimes given readings on the border-line between susceptibility and resistance. Both, however, have given "4" type reactions in warm, sunny weather, and this is considered to be a truer criterion of their infection capabilities than readings obtained in dull, cool weather. Whether or no, however, seven experiments with each variety, carried out at different seasons of the year, give average readings of "3–" and "3." On this basis, therefore, Marquis and Arnautka have been included amongst the varieties susceptible to K 4 in Table I.

Accepting the values given in Table I, no form corresponding to the readings for form K 4 could be discovered in a key to forms 1–100 kindly supplied by Prof. Stakman of Minnesota. When this difficulty was mentioned to him, he drew my attention to the data given by Newton and Johnson in Canada ((3), p. 27) for form 116. The classification of the American differential hosts as resistant or susceptible to forms 116 and K 4 is the same for each variety, but the agreement in mean readings leaves something to be desired in one or two instances, as is shown in Table II, in which Newton and Johnson's data are quoted. Their reading for Kanred is taken as comparable with the Kenya reading for Reliance.

Table II. *Mean reactions of wheat stem rust differential hosts to forms* 116 (*Canada*) *and K* 4 (*Kenya*)

	Physiologic form	
Variety	116	K 4
Little Club	4	4–
Marquis	4–	3–
*Kanred	0	—
*Reliance	—	1–
Kota	3	3–
Arnautka	4	3
Mindum	4	4+
Spelmar	4	4+
Kubanka	4	4–
Acme	4–	3
Einkorn	1	1=
Vernal	4–	3++
Khapli	1–	1++

* Interchangeable.

Special interest attaches to the question of the identity of the Canadian form 116 and the Kenya form K 4, because the former was produced artificially by hybridisation and is believed not to have been found in nature, whereas K 4 was obtained from a field collection from a natural infection.

The distribution of forms K 3 and K 4 so far recorded is given in Table III.

Table III

Locality	Altitude in ft.	Date of collection of rust sample
Form K 3:		
Njoro	7100	Sept. 1930
Mau	8500	Dec. 1930
Nairobi	5700	June, 1931
		Aug. 1931
		Feb. 1932
Iringa	6200	Sept. 1932
Form K 4:		
Ulu	4500	June, 1931
		July, 1931
Nairobi	5700	Aug. 1931
		Feb. 1932
		Feb. 1933

Iringa is in Tanganyika Territory, 8° south of the equator. The other localities are all in Kenya Colony and their position has been described either in (1) or earlier in the present paper. They lie between the equator and 2° south of it.

Summary

The occurrence of a third and fourth physiologic form of wheat-stem rust in Kenya Colony is recorded, together with the data on which they are differentiated from forms previously known to exist in the country. These forms are known locally as K 3 and K 4.

Variations in the climatic conditions under which the investigations were conducted at different seasons of the year led to difficulties in the evaluation of the degree of resistance or susceptibility of certain of the American differential hosts used in distinguishing the forms. The effect of this on the degree of reliability with which Kenya forms can be identified with those recorded elsewhere is discussed.

The opinion is expressed that Kenya form K 3 may be confidently identified with that known in America as form 34. Kenya form K 4 exhibits a close general similarity to form 116, produced artificially in Canada by hybridisation, but the question of their complete identity cannot be considered definitely settled.

The distributions of forms K 3 and K 4 to date are given.

I desire to acknowledge gratefully the assistance received from the various persons already mentioned in this paper and from Mr F. W. Evans, my laboratory assistant, who carried out the greater part of the inoculation work and actually first detected the presence of form K 3 in the rust cultures.

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STUDIES IN THE MORPHOLOGY AND BIOLOGY OF *HELMINTHOSPORIUM AVENAE*

By R. W. G. DENNIS, B.Sc.

(With Plates XII–XV and 2 Text-figures)

Since the year 1918 work has been in progress at the West of Scotland Agricultural College under the direction of Dr D. G. O'Brien, on the control of the leaf-stripe disease of oats. In the course of this work I have carried out investigations on the morphology and biology of the causal agent: *Helminthosporium Avenae* Eidam. Such of the results already obtained as are more particularly of mycological interest are here presented.

Historical review

Briosi and Cavara in 1889 appear to have been the first to observe the presence of a species of *Helminthosporium* on oats, and they described it under the name *H. teres* Sacc. forma *Avenae sativae*. Eight years later Eidam carried out infection experiments from which he concluded that the forms attacking barley and oats were distinct species and gave the name *Helminthosporium Avenae* to that on oats. In the present century little attention has been paid to the leaf-stripe disease of oats, although considerable advances have been made in our knowledge of the similar troubles in barley due to *H. gramineum* Rabenhorst and *H. teres* Sacc. In 1900 F. Kolpin Ravn published a monumental account of his researches into the species of *Helminthosporium* attacking barley, and included notes on the related disease of oats. He described the symptoms of the latter in some detail, isolated the pathogen and experimentally demonstrated its inability to attack barley or rye. In Ravn's view, however, *H. Avenae* was of little economic importance in Denmark at that time. Even as late as 1923, Drechsler in his studies on the graminicolous species of *Helminthosporium* says, in speaking of the oat disease, "As Leaf Spot usually is not a serious disease, and in the United States not very conspicuous in its manifestations, little attention has been paid to its control." It should be noted, in passing, that "leaf spot" is not a satisfactory name for this disease, being most applicable to those attributed to manganese deficiency, and to attack by *Leptosphaeria Avenae*. The term "leaf stripe," therefore, was suggested by O'Brien and Prentice in 1930. Within recent years, however, it has become apparent that, in the

north of Europe at any rate, the leaf-stripe disease of oats is capable of causing serious financial loss to the farmer. The most important recent paper on the subject is that by Rathschlag(10), who had the good fortune to obtain the perfect stage of the oat fungus in pure culture, and succeeded in reproducing leaf-stripe symptoms on oats by inoculation with ascospores. As had long been anticipated, the perfect stage proved to be a species of *Pleospora*, and Rathschlag named it *Pleospora Avenae*. Since, however, perithecia have not been recorded in this country and all references in the literature are necessarily listed under the conidial form, I have preferred to adhere to the more familiar name in the present paper.

In 1931 there appeared a paper by Turner and Millard which consists in the main of a recapitulation of facts previously published by Rathschlag(10) and by O'Brien and Prentice(9). In several respects, however, Turner and Millard's findings differ radically from those here recorded, and will be further discussed under the appropriate headings.

Etiology

Symptoms of *Helminthosporium* attack on the oat plant occur in two sharply defined periods of its life history, the first on the seedling, and the second on the mature plant. These are separated under field conditions by a disease-free period, and will be referred to as the primary and secondary phases respectively.

(1) *Primary phase*

The first organ of the oat seedling to be invaded by mycelium of *H. Avenae* is the coleoptile which, in an infected plant, exhibits one or more narrow brown longitudinal lesions. The subsequent development of the seedling depends upon the intensity of invasion by the parasite. In severe cases the young shoot is attacked before it emerges from the tip of the coleoptile. Such plants are so weakened as to be unable to penetrate the overlying soil; they assume a contorted etiolated form and finally die (Pl. XII). This hitherto undescribed phase of the disease is responsible for the poor stands ("Braird") obtained in the field in crops grown from infected seed. Similar "pre-emergence blight" occurs in other cereal diseases, for example those caused by *Fusarium culmorum* on oats(12) and *F. nivale* on rye.

Injury to the root system by *Helminthosporium Avenae* has not been observed, nor is there any definite record of it in the literature. Melchers(8) records *Helminthosporium* associated with *Fusarium* and other fungi in a foot-rot of oats in U.S.A., but the species is not specified. In view of the prevalence in America of *Helminthosporium sativum*, a common cause of foot-rot of cereals, it is perhaps permissible to infer that this was the species involved. Turner and Millard(16) make no mention of foot-rot or root injury in their discussion of the

symptoms of the oat disease, but in their section "Histology of the Infected Seedling," after recording entrance of the parasite through scutellum and epiblast, they state: "From these points the fungus spreads upwards to the cells of the coleoptile, inwards towards the rudimentary coronal roots, and downwards to the seminal roots, where it may produce the 'foot-rot' of American workers." No evidence is presented to show that the fungus observed in this situation was *H. Avenae*.

The fate of seedlings which, although infected, succeed in emerging above ground is determined partly by intensity of attack, and partly by the weather conditions prevailing and the consequent rate of growth of the host. If the first true leaf is completely killed before the second leaf emerges then the seedling dies. More frequently the first true leaf merely exhibits a narrow brown stripe extending upwards from its base and usually not reaching the tip (Pl. XIII). The diseased tissue may occur along one edge of the leaf, which is then usually distorted, or, more frequently, may be isolated in its centre, when no distortion results. In seedlings of this class the second and third leaves usually exhibit a leaf stripe similar to that on the first leaf, but after the emergence of the fourth leaf all trace of the disease vanishes for the time.

Finally, under conditions highly favourable to the growth of the host, the attack does not proceed beyond the coleoptile. It is fair to say, therefore, that the coleoptile stripe is the only phase of this disease which is invariably present.

(2) *Secondary phase*

Late in July or early in August, *Helminthosporium* attack reappears in the field on the upper leaves of the oat plants. It here takes the form of minute brown flecks which gradually elongate and develop into more or less well-defined longitudinal stripes of dead tissue.

As regards damage done to the crop, the primary phase is the more serious of the two. The loss of two or three leaves at the most critical period of its life history, namely that of tillering, causes the plant to remain stunted throughout. It becomes overshadowed by its healthy neighbours, its tillers do not develop, and when the ear emerges the basal spikelets are barren. This sterility is not due to invasion of the flowers by mycelium of *H. Avenae*, but is merely a symptom of the generally weak and semi-starved condition of infected plants.

Morphology of the fungus

(1) *Mycelium*

Pure cultures of *H. Avenae* may be readily isolated from any of the types of lesions outlined above, or may be obtained by plating spore suspensions. The mycelium grows well on any of the ordinary media,

4 per cent. oatmeal agar, 4 per cent. maize meal agar, and 2½ per cent. malt agar having been mainly used in the present investigations. On these substrates strains of *H. Avenae* are characterised by a more or less profuse development of pure white aerial tufts (Pl. XV, fig. 1) which show up clearly against the greenish grey or almost black mycelial mat. The submerged mycelium rapidly appears almost black in the mass, due in part to the development of brown pigment in the agar, and in part to the darkening of the hyphal walls with age, and production of chlamydospores. On malt agar some strains show a definite tendency to the development of pink and purple crystalline and dendritic deposits in the medium, which lend a faint pinkish aspect to the cultures when viewed from below. A few strains, when grown in the light, exhibit diurnal zoning, but this cannot be regarded as characteristic of the species.

Considerable variation exists between the appearance of different strains of *H. Avenae* growing in pure culture, there being especially a tendency to vary in the direction of greater or lesser production of aerial tufts. As usual the strains exhibit a tendency to saltation, but since *H. Avenae* presents no new feature in this respect and since the phenomenon has been fully studied in *H. sativum*, and other species by Stevens (15) and Christensen (2), it will not be further considered here.

On germination of a spore of *H. Avenae* a mycelial network is produced consisting of thin-walled, colourless, septate hyphae filled with a vacuolate, finely granular cytoplasm. The component cells are at first about 5–7 μ in width and at least 20 μ long. Each contains a number of nuclei which apparently divide mitotically, although they are too small for the details to be made out. Occasional anastomoses occur between different hyphae. As the culture ages the old submerged hyphae increase in width, and become more closely septate, finally developing into short chlamydospores with fairly thick brownish walls (Pl. XIV E) and conspicuous oil globules. Chlamydospores may occur in short chains separated by empty unaltered cells. At the same time the aerial hyphae undergo a process of auto-digestion. This commences at the base of the tufts, causing them to fall over on to the surface of the medium, and eventually involves the entire aerial mat.

(2) *Conidia*

Turner and Millard (16) report complete absence of spore production on any artificial medium, including tap-water agar. It should perhaps be mentioned that a culture labelled as *H. Avenae* was obtained from these workers and found to be completely unlike any of the numerous strains isolated in the present investigation. It remained sterile throughout a number of transfers and was discarded, the evidence of its identity being considered incomplete.

In all other strains met with conidia are produced sparingly on most media, but can be obtained in abundance when the fungus is grown on tap-water agar, as recommended by Drechsler (4). A conidiophore may develop from any hypha, which then sends a branch up from the surface of the agar, and develops a conidium at its apex. Growth of the conidiophore is resumed in a lateral direction, a second spore produced, and so on. The mature organ, therefore, exhibits a series of knee-shaped bends, each marking the point of attachment of a conidium (Pl. XIV A, C). The whole process has been already clearly described by Ravn (11). Less frequently, conidia may be developed in a catenulate manner, as has been described by Stevens (15) in *H. sativum*. On diseased oat tissue* the conidiophores project at random through the epidermis, either alone or in groups. Here they are thick-walled and brown and the basal cell is somewhat swollen, affording a firm point of attachment to the epidermis.

The conidia themselves are approximately cylindrical† with rounded ends and a yellowish brown, the colour deepening with age. The wall is composed of a thin brown episporium, about $0{\cdot}5\mu$ thick, enclosing a colourless endosporium which alone participates in the formation of septa. In young conidia the endosporium is about 1μ thick, but may become greatly thickened with age. Each conidium consists of a number of cells varying from one to thirteen (usually from four to nine), which act as independent units. In old conidia some cells die earlier than others, and if such spores are germinated, a germ-tube may occasionally be extruded from a living cell into the lumen of its dead neighbour. The basal end of each conidium is marked by a black spot, representing an air space, but there is no indication of light spot at the apex, as in *H. sativum* (15).

Tri-pointed spores, such as those recorded and figured by Turner and Millard (16), have never been observed by me, either on foliar lesions or in pure culture. As the conidial dimensions recorded below involved the measurement of 1800 spores, without the discovery of a single tri-pointed conidium, it is evident that these cannot be regarded as characteristic of *H. Avenae*.

In germination the episporium is ruptured, the endosporium protrudes over a sharply defined circular area, and develops into a germ-tube. There is no indication of germ-pores in the resting conidium and germ-tubes may be protruded from any point of the wall, with the exception of the black basal spot. Usually, however, the end-cells germinate first.

In instituting comparisons between spore dimensions it is essential that all spores examined should have been developed under uniform

* Conidiophores are developed on the dead central portion of the lesions.

† The Coefficient of Cylindricity, determined according to the criteria laid down by Stevens (15), was ·928 for fifty conidia.

conditions. This is especially so in the present genus. The mean length of 500 spores produced in the field in September, 1931, was 85μ, that of the same number developed on infected leaves kept in a moist chamber was 106μ. The dimensions of conidia produced on artificial media are invariably smaller: those of strains A and C on maize-meal agar being 64 and 60μ respectively. Strain C was the only one dealt with which produced pycnidia. Moreover, its growth in culture was characterised by the poorest development of tufts, so that it is important to observe that as regards structure and appearance of conidia it proved practically identical with the standard strain A and must be regarded as belonging to the same species. Mean spore width varies from 20 to 30μ.

(3) *Resting mycelium and sclerotia*

On the husk of infected oat grain a thick-walled resting mycelium is developed. It consists of pale brown hyphae composed of short cells of irregular outline about 7μ wide, and frequently with interlocking end-walls. Similar resting mycelium has been described and figured by Vogt(17) in *H. gramineum*. Small knots, more or less compacted, occur at intervals and may be regarded as imperfect sclerotia. True sclerotia occur less frequently, sunk in the host tissues on the flanks of vascular bundles (Pl. XIV L, M). They are visible to the unaided eye as minute black dots and are spherical or elliptical. In section they are seen to consist of a distinct outer layer of dark brown, thick-walled papillate cells, enclosing a colourless thin-walled, compact pseudo-parenchyma full of oil globules. Similar bodies develop in pure culture, especially among mycelium in contact with the glass. In size they vary greatly, between 50 and 200μ long. Larger examples seen in culture are irregular in form and probably arise from the union of two or more smaller ones.

(4) *Pycnidia*

Pycnidia of *H. Avenae* have apparently not hitherto been described. During the present work they were obtained in one culture only, the first isolation of strain C from secondary infection of oats. Subcultures of this strain, though apparently identical in all other respects, have never yielded pycnidia. It is interesting to note that Kohl(7), working with *Phyllosticta solitaria* E. & E., was similarly able to obtain pycnidia only in cultures containing host tissue. In his experience also "Subcultures obtained by making mycelial transfers failed to sporulate." The pycnidia are first visible to the naked eye as minute black spots. They are of the *Phoma* type, having a brown, opaque wall, one cell thick, with an irregular apical opening from which pycno-conidia were extruded in a compact roughly spherical mass (Pl.

XIV K). The spores were minute, unicellular, and hyaline, measuring $2 \times 2-3\mu$. All attempts to germinate them failed.

The above description tallies almost exactly with that given by Ravn(11) for pycnidia of *H. teres* on barley straw, and affords further evidence of the extremely close relationship between the two species, which has been emphasised by most workers from Briosi and Cavara onwards.

(5) *Perfect stage*

Although a careful search has been made for the past three years the perithecia of *Pleospora Avenae* have not been observed in Scotland in the field, nor have they developed in pure culture.

Rathschlag(10) obtained them once only, and *in vitro*, and it appears safe to assume that, like pycnidia, they play no part in the normal life cycle of the fungus. It should be mentioned that Ito(6), in Japan, has obtained perithecia on oat straw which he claims belong to *Helminthosporium Avenae*. His description, however, differs in important respects from that of Rathschlag, and it is probable that his findings are inapplicable to European conditions.

Pathogenicity

The pathogenicity of *H. Avenae* to oats has already been satisfactorily established. In the present work, however, in view of the large number of strains accumulated from all parts of Scotland, it was considered desirable to test some of the more distinct of these in order to determine if all were capable of parasitising oats to the same extent. The method adopted was as follows: Two sets of seed, fifty grains in each, were taken at random from a sample tested and known to be practically disease-free. In one of these a small portion of an agar culture of the strain to be tested was inserted between the tips of the paleae. In the companion set, which served as controls, pieces of sterile agar replaced the culture. The two lots were then sown in boxes of soil and left in the open air. Results obtained with seven strains in the summer of 1932 are summarised in Table I.

Table I. *Inoculation of grain with strains of* H. Avenae

		Strain							
		5	14	57	59	83	91	95	Control
Exp. 1	Braird %	68	80	58	56	80	—	82	82
	Leaf stripe % ...	17·9	5·0	34·5	25·0	10·0	—	17·0	0
	Coleoptile stripe %	56	48	46	48	42	—	70	2
	Contorted seedlings	22	8	22	34	14	—	18	0
Exp. 2	Braird %	54	76	80	92	80	78	—	92
	Leaf stripe % ...	33·3	10·3	25·0	24·0	7·5	12·8	—	0
	Average leaf stripe %	23·3	8·8	28·3	18·9	9·6	12·8	17·0	0

In Exp. 1 the seedlings were all dug up after eighteen days and examined for coleoptile stripe and contortion. In the second experiment they were allowed to grow to maturity. Percentage leaf stripe is calculated on the "braird," *i.e.* on the number of seedlings which appeared above ground; the other percentages are calculated on the number of seeds sown. Amongst others the following outstanding conclusions may be drawn:

(1) Contamination of oat seed with mycelium of *H. Avenae* is sufficient to result in a marked decrease in braird.

(2) Inoculation of grain with a pure culture of this fungus is sufficient to produce all the symptoms of the primary phase of leaf-stripe disease. The contorted seedlings illustrated in Pl. XII were obtained in the first experiment of Table I.

(3) Different strains of *H. Avenae* may show marked differences in degree of pathogenicity to oats. Although the figures obtained in the two experiments are not in exact correspondence, yet the strains employed clearly fall into two distinct groups—one of high pathogenicity, represented by numbers 5, 57, 59 and 95, and one of low pathogenicity, containing types 14, 83 and 91.

Similar differences in pathogenicity between strains of *H. gramineum* are recorded by Christensen and Graham (3).

Conidial infection

In carrying out infection experiments with conidia of *H. Avenae*, oat leaves were enclosed within Petri dishes containing moist cotton-wool, without being detached from the plant. Usually the middle portion of a leaf was employed, the base and tips being left outside the dish. The surface to be inoculated was placed uppermost, moistened, and sown with conidia. Under these conditions the lesions characteristic of secondary infection were readily developed. Minute flecks appeared on the leaves after four days, and by the end of a week these had become enlarged into brown stripes about a quarter of an inch long, with yellowish margins of recently invaded tissue. Controls, set up in a similar manner, but without conidia, remained healthy.

Conidia germinate readily in drops of water on leaf surfaces. The germ-tubes grow a short distance, usually not more than the length of the parent spore, and form small round terminal appressoria (Pl. XIV G, H, J). They do not seek out the stomata but usually, if a pore is encountered, an appressorium is formed over it (Pl. XIV F, G). A hypha grows from this between the guard cells and forms a slight swelling in the substomatal chamber. Thence a normal hypha develops and finds its way between the adjacent host cells. When the appressorium is produced over the uninterrupted cuticle a thin penetration hypha is protruded which swells to normal dimensions

on attaining the cavity of the epidermal cell. The latter is traversed by the shortest route, and a slight swelling is developed when the hypha impinges on the inner wall. This is penetrated and subsequent growth of the mycelium is restricted to the intercellular spaces of the mesophyll. If penetration fails the germ tubes resume growth, and develop into a network of greatly attenuated hyphae, following the slight furrows which mark the outline of the epidermal cells.

Where leaves were allowed to dry within twenty-four hours after inoculation penetration took place, but growth of the hyphae within the leaf was checked by the formation of brown deposits where their tips were insinuated among the palisade cells (Pl. XIV, F–J). The importance of the part played by atmospheric humidity will be further stressed when the origin of secondary infection is discussed (p. 235).

Influence of temperature on mycelial growth

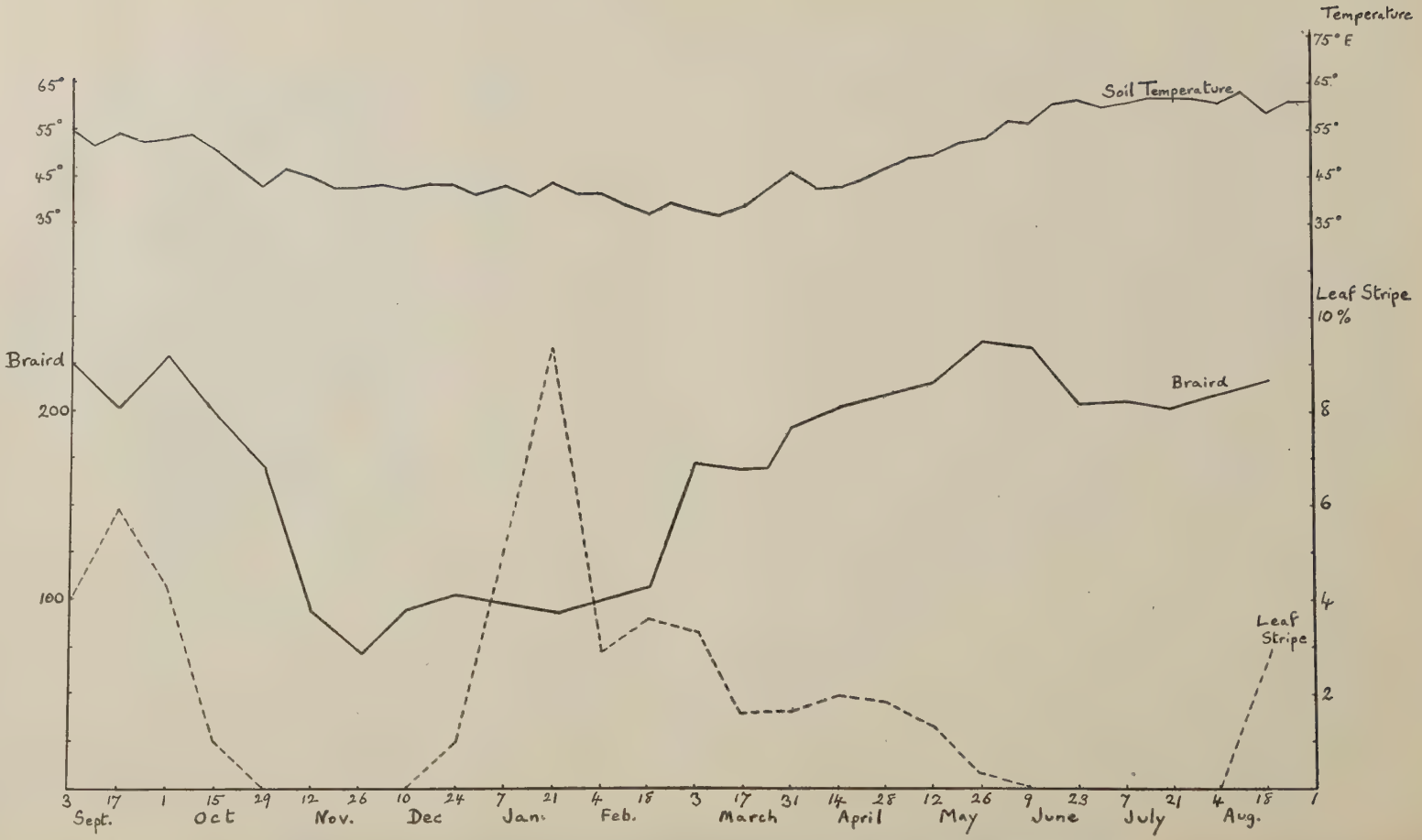
Rathschlag(10) reports that the optimum temperature for the growth of *H. Avenae* in pure culture lies between 20 and 25° C., the maximum and minimum temperatures being 30 and 2–3° C. respectively. He also states that mycelium can withstand a temperature of − 14° C. for at least fifty-six days. I have been able to confirm in part these figures, as shown by the data presented in Table II.

Table II. *Diameter of cultures of* H. Avenae *grown at various temperatures*

Time	17° C. mm.	20° C. mm.	25° C. mm.	28° C. mm.	30° C. mm.
2 days	8·5	10·0	9·0	4·75	3·0
5 „	25·0	26·5	24·5	9·0	4·5
10 „	62·0	70·5	65·0	25·0	4·5

The above figures represent the average diameters of a duplicated series of cultures growing on oatmeal agar in the dark. It will be seen that an optimum of about 20° C. and a maximum at slightly over 30° C. are indicated.

The influence of the temperature factor is most strikingly seen when the relation is studied between date of sowing of infected grain and intensity of primary infection. Samples of three hundred grains each, from a diseased stock of pure line Potato oat, were sown at fortnightly intervals at Auchincruive, Ayrshire, from September 3rd, 1931, to September 1st, 1932. The seeds were planted individually three inches apart, in a bird-proof cage, to facilitate counting after germination. Graphs of the results obtained are supplied in Text-fig. 1. It will be observed that there are two maxima for seedling infection, namely in January and September. There are also two periods during which disease does not appear, namely in sowings over



Text-fig. 1. Relation between date of sowing and incidence of leaf-stripe disease on oat seedlings.

a period of six weeks, October–December, and from the beginning of June to the end of August. Of these the winter period is one where braird and leaf stripe decline together. Temperature is low and humidity high, conditions known to be favourable to the growth of *H. Avenae* and relatively unfavourable to the development of seedling oats. At this time all such plants as are attacked by the leaf-stripe fungus succumb before reaching the soil surface. In the succeeding zone of the graph—that of seed sown in late winter and early spring, and germinating in spring, braird increases and the above-ground manifestation of disease reaches a maximum. Here environmental conditions are still favourable to the parasite, but are less unfavourable to the germination and establishment of the host. The seedlings grow quickly, and a number succeed in reaching the surface of the soil and developing more or less normally in spite of attack by the fungus. Later in the season, as the temperature of the surrounding soil gradually rises, the balance swings more and more in favour of the host until, in June sowings, braird reaches a maximum, and disease disappears.

In autumn this course of events is reversed, but it will be observed that, whereas disease does not disappear until midsummer, its re-appearance is delayed until September. This is doubtless related to the relatively slow heating of the soil in spring, and its delayed cooling in autumn.

The host-parasite relationship

Source of the disease

All attempts at the control of leaf-stripe disease of oats have been based upon the assumption that the infection is carried over winter on the seed. Rathschlag (10) was unable to find hyphae in endosperm, scutellum, embryo or pericarp of infected oat grain and concludes that the only sources of primary infection are through conidia adhering to the grain and through mycelium existing between the pales and the kernel. Hence, thorough seed disinfection provides an efficient method for control of leaf-stripe disease on the seedling, and very satisfactory results on these lines have been obtained in Scotland in recent years. It is hoped to publish a full account of this work in the near future, in the form of a Research Bulletin of the West of Scotland Agricultural College.

There remained, however, the possibility that a further source of infection might exist in the soil of fields from which an infected oat crop had been taken. Colour was lent to this supposition by the reported experience of certain farmers that when oats are grown on the same land in consecutive years the disease is more severe in the second year. Experiment has shown, however, that although the fungus grows freely on sterilised soil, it is unable to establish itself on

unsterilised soil. Moreover, when plates of sterilised soil are inoculated at opposite ends of a diameter with fragments of a culture of *H. Avenae* and with minute portions of unsterilised soil, the fungus proves unable to compete with the normal soil flora, and no growth takes place.

A further possible minor source of disease occurs where straw or stubble bearing sclerotia of *H. Avenae* is ploughed in before sowing, but in view of the above experiments it would appear that this must come in actual contact with the grain in the drill if infection is to result.

Development of primary infection

Smith (13) has furnished a valuable and detailed account of the host parasite relationship in *H. gramineum*. As far as the primary phase is concerned *H. Avenae* exhibits a closely parallel development. Resting mycelium, if present, is usually to be found on the tips of the paleae, as is to be expected from their position in the flower, where these form the portion of the grain most liable to catch wind-blown spores. As the grain germinates, the coleoptile emerges between the tips of the paleae and, owing to curvature of the outer pale, it usually comes in contact with them only at two points, one on each side. If mycelium of *H. Avenae* is present on the tips of the husk, and has been rendered active by favourable environmental conditions, it will attack the emerging shoot at these two points. As the coleoptile grows, fresh portions of its surface are exposed to the infective areas, and the result is a narrow longitudinal stripe of diseased tissue proceeding upwards from each point of contact with the husk (Text-fig. 2). Less frequently a third stripe is produced, corresponding with the tip of the inner pale, which touches the coleoptile midway between the edges of the outer pale.

Text-fig. 2. Infection of the seedling. *l*, coleoptile lesion.

Turner and Millard (16) present evidence to show that infection takes place as soon as germination begins. "On germination the dormant mycelium spreads towards the embryo, and penetrates it at those points where the cells offer the least barriers, *i.e.* at the junction of the scutellum and the epiblast." Doubtless the course of events envisaged by these workers affords a ready explanation of those cases in which the coleoptile never emerges. Under field conditions, however, the greatest death rate from the leaf-stripe disease occurs

among the so-called contorted seedlings (Pl. XII) in which the coleoptile lesions are arranged in one or two thin longitudinal lines, which can be traced downwards to their origins at points of contact with the pales. Turner and Millard do not appear to be aware of the existence of this important phase of the disease, and from their interpretation of the initiation of disease it is difficult to explain the peculiar and constant form of the coleoptile lesions, or the remarkably complete control obtained by the use of dry disinfectants.

The mycelium quickly penetrates the soft parenchyma of the coleoptile, and grows freely in the moist atmosphere enclosed. Here it comes into contact with the first leaf, as yet closely rolled upon itself, so that only a narrow longitudinal strip is exposed to the parasite. Consequently, when the first leaf expands, the infected tissue appears either as a single longitudinal stripe or as a number of spots arranged in a single line. The second leaf is similarly infected while within the sheath of the first and so on. In *H. gramineum* (13) the fungus passes thus from leaf to leaf, and finally reaches the ear. In *H. Avenae*, on the contrary, a disease-free period sets in with the emergence of the fourth leaf. The exact reason for the interpolation of this healthy period is not at first sight easy to understand, but a number of possible contributory factors may be adduced. In order for primary infection to persist it is necessary that each leaf should remain within the sheath of its predecessor long enough to be penetrated by mycelium growing in the enclosed air space, *i.e.* the host must be growing slowly. The period of tillering, which lasts until about the expansion of the fourth leaf, is one in which growth of the main stem is almost suspended, the resources of the plant being expended on the initiation of lateral shoots. Moreover, this period falls in spring, when the temperature is such as to favour the growth of the mycelium rather than that of the host. As weather conditions improve with the advancing season, the crop (tillering completed) grows quickly, leaves emerge and unfold and internodes elongate far more rapidly than at first. Under these circumstances the host develops faster than the parasite, which becomes out-distanced and stranded in the base of the stem.

Origin of secondary infection

That the secondary infection of oats by *H. Avenae* which appears in July and August under Scottish conditions is not merely a delayed aspect of the primary phase is shown by the following considerations:

(1) There is an interval of some six weeks between the cessation of the primary and the beginning of the secondary phase. During this period the upper leaves, numbers 5–7, expand and remain healthy.

(2) The disease reappears as minute scattered spots, each arising

independently of the others, and not arranged in lines parallel with the long axis of the leaf.

(3) Except very rarely no lesions can be detected on the leaf sheaths which enclosed the upper leaves prior to their expansion. No mycelium can be detected within the tissues or on the inner surface of such sheaths at the time when secondary infection first appears.

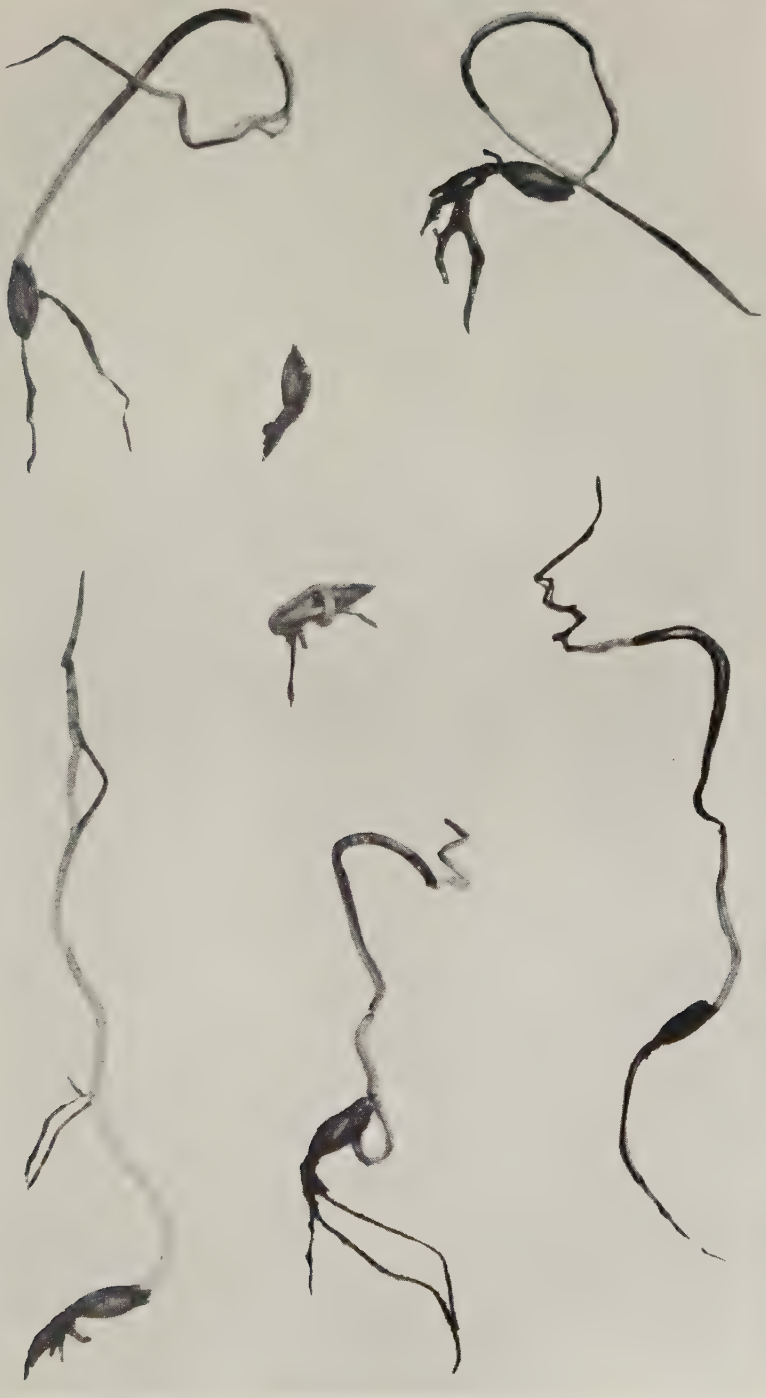
(4) In a series of oat plants carefully examined each week from the seedling stage until harvest it has been observed that certain of those recorded as escaping the primary phase of the disease exhibited the secondary phase earlier than those noted as infected from the seed.

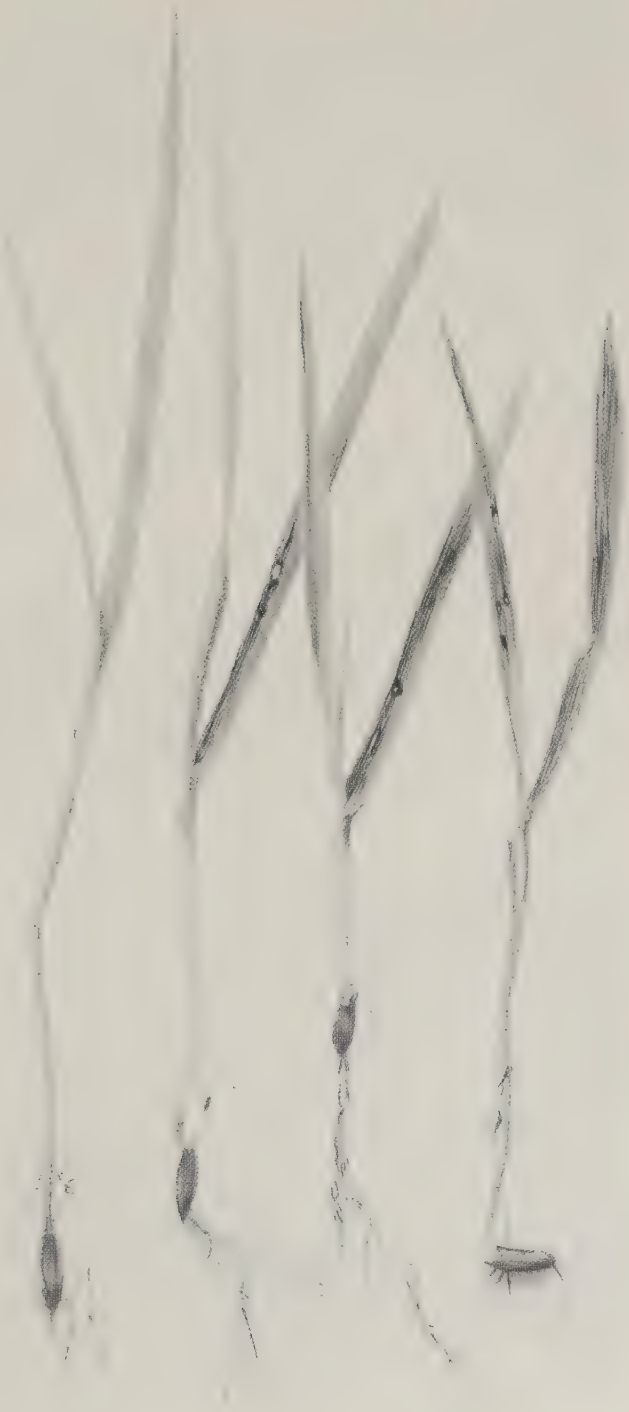
(5) Crops in which primary infection was eliminated by efficient seed disinfection may be just as heavily infected by the secondary phase as an untreated crop. In an experiment at Auchincruive in 1931 seed treatment proved very effective, but examination of eighty leaves taken at random in September showed 93·7 per cent. to be infected by *H. Avenae*.

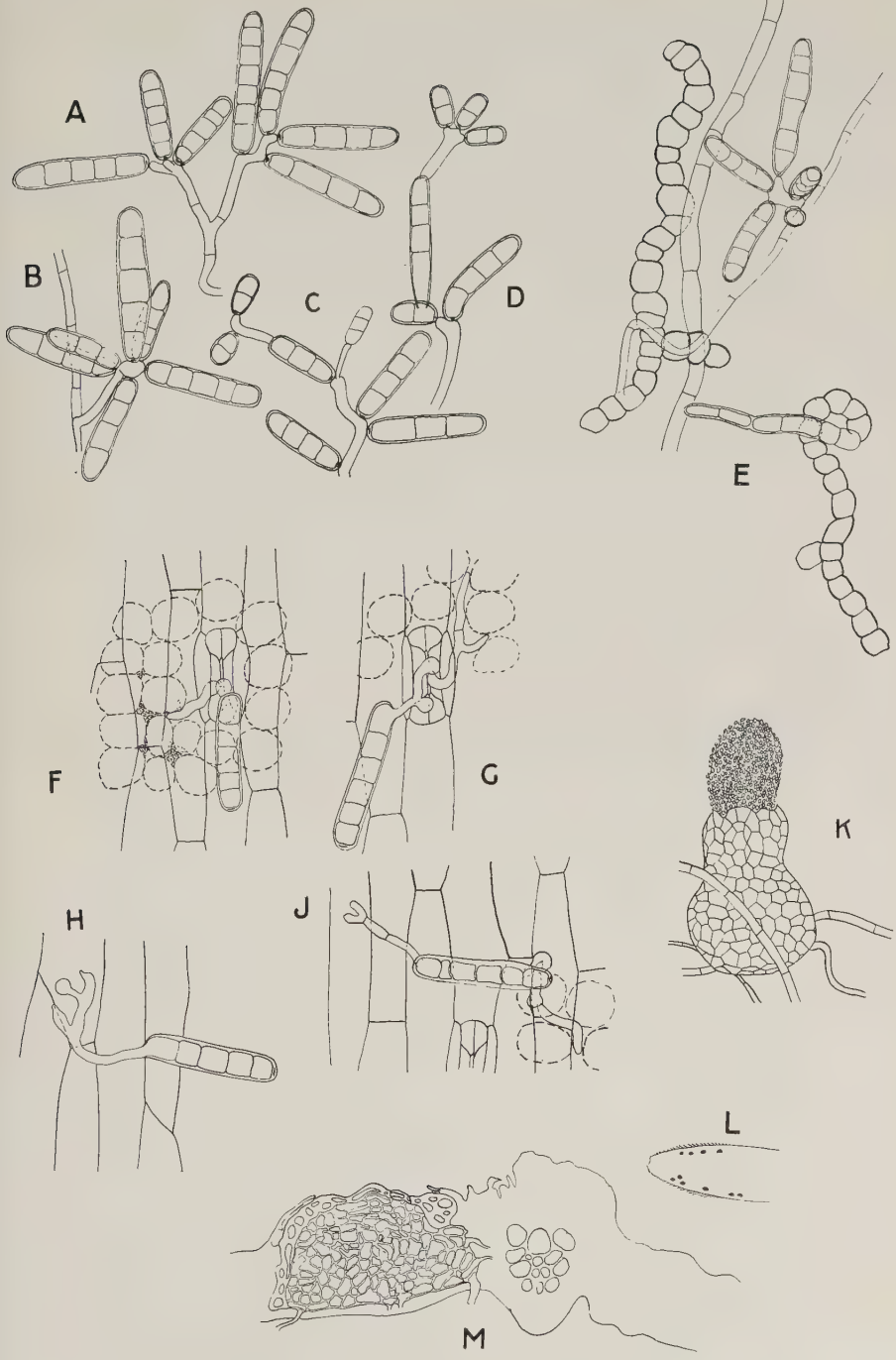
(6) The characteristic symptoms of the secondary phase can be reproduced artificially by sowing conidia on the leaves.

It is therefore concluded that the secondary infection of oat plants by *H. Avenae* is due to the germination on the leaves of wind-blown spores derived from tissues killed by the primary attack. Spores of the *Helminthosporium* type are spread readily by the wind; they have been recovered from the air at heights up to 10,000 ft. (14) and consequently the disease may spread rapidly from field to field. The non-appearance of conidial infection until late summer is probably to be attributed to the difficulty of maintaining sufficiently humid conditions in the crop to permit of penetration of the epidermis by germ-tubes. Such conditions do not obtain amongst cereal crops in the field until the plants have attained a height of $2\frac{1}{2}$–3 ft., and until all the leaves have expanded. Then, however, the crop, forming a more or less compact cover over the ground, responds to climatic conditions as a unit, and the drying influences of sun and wind are restricted to its upper surface and to its edges. It is only then that conidial infection becomes possible and that the first signs of secondary infection appear. Plants widely spaced, or placed where they are continually disturbed by wind, so that a relatively dry atmosphere is maintained around them, never develop disease beyond the seedling phase, even on the most decrepit leaves.

From the above observations one may conclude that leaf stripe of oats represents a disease in which the relationships of parasite and host are intimately controlled by the environment. The primary phase is limited by temperature, and the secondary phase by atmospheric humidity, while the intermediate healthy period is made possible by a combination of dry atmospheric conditions with temperatures relatively favourable to the growth of the crop.



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Summary

1. A description is given of the symptoms of a disease of oats caused by *Helminthosporium Avenae*, and popularly known as "leaf stripe." They are classified according to the season of occurrence, as belonging to the primary phase on the seedling, or the secondary phase on the mature plant.

2. The morphology of the fungus is described in outline, under the heads: Mycelium, Conidia, Sclerotia and Resting Mycelium, and Pycnidia, the latter not hitherto described.

3. The optimum temperature for growth of *H. Avenae* is shown to lie about 20° C. and the maximum about 30° C. In the field the primary phase of the disease is limited largely by temperature, reaching a maximum on the leaves in the autumn and early spring.

4. All stages of the disease have been successfully reproduced artificially in inoculation experiments with pure cultures of the fungus.

5. The source of disease each year lies in the grain. *H. Avenae* cannot exist saprophytically in the soil.

6. The primary phase follows closely the lines indicated by Smith in *H. gramineum*.

7. The secondary phase is not a direct continuation of the primary, but originates from wind-blown conidia and depends for its initiation upon the maintenance of a sufficiently high atmospheric humidity in the crop.

EXPLANATION OF PLATES XII–XV

Plate XII

Contorted oat seedlings, obtained by inoculation of grain with *Helminthosporium Avenae*.

Plate XIII

Leaf stripe of oats, the primary phase.

Plate XIV

Morphology of *Helminthosporium Avenae*. A–D, conidia and conidiophores (C–D, catenulate conidia) from tap-water agar (× 250); E, mycelium with chlamydospores (× 250); F–J, penetration of oat leaves by germ tubes (× 250); K, pycnidium, strain C (× 175); L–M, sclerotia on inner pale of oat grain (L, × 2; M, transverse section of pale, × 250).

Plate XV

Cultures of *Helminthosporium Avenae* on oatmeal agar. Fig. 1, strain 57; Fig. 2, strain C.

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A SYSTEMIC DISEASE OF RED CLOVER CAUSED BY *BOTRYTIS ANTHOPHILA* BOND.*

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(With Plate XVI and 2 Text-figures)

I. Introduction

During breeding work at the Welsh Plant Breeding Station an interesting disease of red clover has been observed in which the pollen grains in the anthers of infected plants are replaced by the spores of a species of *Botrytis* (Pl. XVI, fig. 1).

On diseased plants the anthers present a fluffy ash-grey in place of the normal bright yellow. The symptoms are evident even in very young buds. Usually all the florets on every head on a diseased plant are infected, but occasionally some heads contain both yellow and grey anthers. Infected plants exhibit no external signs of disease, differ in no obvious way from normal healthy individuals and appear to be quite as vigorous.

The fungus has been isolated and studied in pure culture and agrees with *Botrytis anthophila* described by Bondarzew (1913).

II. Occurrence

The disease has been reported from Russia by Trusova (1912, 1925), Bondarzew (1913, 1914), Jaczewski (1913, 1916) and Lobiz (1922). Schlecht (1921) encountered it in Germany, and Williams (1925) in Wales found that the yield of seed from infected plants was seriously reduced.

Karakulin (1921) reported from 0 to 5·6 per cent. of infected plants in various fields of four varieties of Russian cultivated clovers. Trusova (1925) found that late red clovers were infected to a greater extent than earlies; thus 2·8 per cent. of 3766 plants in six samples of lates from Russia, Sweden and the Baltic region were infected, but only 1·4 per cent. of infection occurred among 1940 plants in eight samples of early red clovers of Russian, Swedish, Baltic and Anglo-American origin.

Field observations were made in 1930 to determine the extent of infection. Many native wilds were investigated *in situ*; Montgomery

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late-flowering types observed were seed crops in Montgomeryshire; other samples were grown at the Welsh Plant Breeding Station from samples of seed received direct from their place of origin. In these plants, observations were made on young unopened florets; this precluded the possibility that the plants had been infected locally during flowering. The disease was observed in material from the following sources:

(1) *Wild red clovers:* England (Cumberland, Yorkshire, Lincolnshire, Kent, Oxfordshire, Devonshire, Gloucestershire, Herefordshire, Shropshire), Wales, Scotland, Ireland, Norway, Sweden, Holland, Germany, Czechoslovakia, Poland, Switzerland, France. Among 2142 plants observed, 85 were infected.

(2) *Early-flowering red clovers:* Broad reds from England, Germany, France, New Zealand, Tasmania; and American Mammoth. Among 492 plants observed, 11 were infected.

(3) *Late-flowering red clovers:* English lates, Montgomery lates, Russian lates. Among 1815 plants observed, 90 were infected.

III. Etiology

An excellent and very full account of the symptoms of the disease and of the fungus itself has been given by Bondarzew (1913, 1914), but this account is not readily available to English readers, and so a short résumé of Bondarzew's observations is given here, together with some confirmations and amplifications which have been made. The dimensions of *Botrytis anthophila* quoted below refer to my material, and they correspond very closely with the dimensions given by Bondarzew.

Microscopical examination of diseased anthers (Pl. XVI, fig. 1) reveals that their ash-grey condition is due to the development on them of the conidiophores and spores of a fungus, which often completely envelop both anthers and stigma. The fungal mycelium ramifies amongst the pollen grains in the anther, and sends out conidiophores through the anther walls. The hyphae in the anthers are very coarse, about 4–6μ in diameter. The conidiophores are almost straight, occasionally slightly wavy or geniculate, rarely with one or two branches along their length, but often branched near their apex. They are 100–200μ long, occasionally up to 500μ, irregularly septate, the cells being from 5 to 70μ long, and 5–8μ in diameter. The apex is somewhat inflated and indentate, about 6·5–10·5μ in diameter, and bears on the swollen ends clusters of up to 18 or 20 ovoid elliptic unicellular conidia which are developed by a process of budding (Pl. XVI, figs. 2, 3). Exceedingly short sterigmata are sometimes visible. Mature conidia range from 9·5 to 20μ (mostly 11·5–15·5μ) long × 3·5–7μ (mostly 4–5μ) broad (Text-fig. 1). Sometimes the conidia are deformed (Pl. XVI, fig. 4). Hyphae, conidiophores and

Text-fig. 1. Frequency graph showing size of conidia.
——— *B. anthophila* (anthers). 200 measurements.
- - - - - - - *B. anthophila* (monosporidial culture on prune agar). 400 measurements.
..... *B. Trifolii* (beerwort). Transcribed from Kingma, 1927.

conidia are colourless. The mycelium is readily visible in the mesophyll of the petal at the base of the corolla tube after staining with cotton blue lactophenol, the hyphae being 6–9μ in diameter (Pl. XVI, fig. 5). The hyphae may be traced into the base of the filament tube where they become thicker, much more frequently septate, swollen (Pl. XVI, fig. 6), and tangled into knots. The mycelium may also be seen in the stem, where the sparse development of the hyphae is restricted to the intercellular spaces of the pith, and is considerably finer than in the parenchymatous tissue of the petal and filaments, being only about 2–3μ in diameter. Extremely rarely the hyphae formed lateral mammiliform haustoria in the medullary cells. No sclerotia have been observed.

Bondarzew has shown that this systemic infection has its origin in an intraseminal mycelium, since seed, even though disinfected, gave rise to infected plants. He showed that the intraseminal mycelium results from the germination of conidia on the stigma at the time of pollination. He also observed the visits of bees to infected plants, and suggested that they probably carry the infection, but did not prove this.

At Aberystwyth, bees were observed working infected heads as diligently as healthy ones, and it was demonstrated experimentally by two methods that bees are often responsible for the distribution of spores. In the first place, bees caught after visiting infected heads were washed with sterile water, the washings were plated out, and the development of the fungus was observed. Secondly, a healthy and a diseased plant were intercrossed by means of a bee in a cage, and of twenty-two progeny derived from the healthy plant, nine were infected with *Botrytis*, as shown by examination of their anthers. In this experiment the conidia, together with pollen grains, must have been carried by the bees from the diseased to the healthy plant.

Spores germinate on the stigmas of infected plants and on the stigmas of healthy plants to which they are carried by the bees, giving rise to very fine hyphae which rapidly traverse the stylar canal in this attenuated condition (Text-fig. 2), and after several days increase in diameter to about 3–4μ. The passage of the hyphae to the ovary cavity and actual infection of the young ovules was traced by Bondarzew (1914), but I did not investigate this.

IV. Cultural characteristics of the fungus

Both Bondarzew (1913, 1914) and Jaczewski (1913, 1916) have studied the growth of the fungus in pure culture, and the following account of its development as observed at Aberystwyth is in agreement with their descriptions. Pure cultures of the fungus are readily obtained from unopened infected florets. Growth on artificial media

is relatively slow, single-spore colonies on prune or malt agar, where the most typical development of the fungus is seen, attaining only to 5 mm. diameter in forty-eight hours, and scarcely exceeding 10 mm. diameter even after several months. On prune agar the initial slender radiating white colony develops to form a closely interwoven very pale olive-green mat below the surface, with a low white tufted growth of aerial conidiophores. Bondarzew describes the development of a

Text-fig. 2. *L.S.* Stigma showing germinating pollen grains and conidia. Stained thionin-orange G. ×380.

rose tint in his cultures, but neither my work nor that of Jaczewski confirms this. Both hyphae and conidiophores are coarse, as in the anthers, ranging from about 2·5 to 10μ in diameter, mostly 5–6μ, irregularly septate, the cells ranging from 50 to 100μ in length, and the terminal expansion of the conidiophores from 5 to 12μ, mostly 7·5–8·5μ in diameter. The conidia are slightly shorter and broader than on the anthers, ranging from 7 to 19μ (mostly 9·5–12μ) long ×4·5–9·5μ (mostly 6–8·5μ) broad (Text-fig. 1).

On Dox's synthetic medium the mycelium is very irregular, very frequently septate into short cells which are often swollen to about twice the diameter of the mycelium, the mycelium being about 2–7μ and the barrel-shaped swellings 8–9μ (Pl. XVI, fig. 7). Spores are formed less abundantly than on prune and malt agars. On peptone agar there is a far less extensive development of mycelium both in and above the substratum; conidia are less abundant, frequently formed below the surface, and a large proportion of them are irregular in shape (Pl. XVI, fig. 4). Small darker green dots are visible in the colonies; these pseudo-sclerotia consist of entanglements of knotted, swollen, frequently septate hyphae, similar to the abnormal type of mycelium which occurs on Dox's medium, and comparable with the ganglia which form at the base of the filament tube. No true sclerotia have been observed in even six-months-old cultures.

V. Taxonomy and synonymy

Bondarzew (1913, 1914) has classified this fungus as a species of *Botrytis*, but Jaczewski (1913, 1916) prefers to regard it as a species of *Oedocephalum* which he has named *O. antophilum*. The frequent branching of the conidiophores and the absence of definite apical swellings are more characteristic of the genus *Botrytis* than of *Oedocephalum*, and I have therefore followed Bondarzew in adopting the name *Botrytis anthophila*. The fungus which Schlecht (1921) described under the name of *B. antherarum Trifolii* is clearly identical with Bondarzew's species.

Kingma (1927) described *B. Trifolii*, a fungus which he isolated from red clover seeds. He does not refer to Bondarzew's fungus, but, from his description, the fungus which he isolated from red clover seed appears to be morphologically similar to the parasite found in the anthers. Thus the mycelium of *B. Trifolii* is described as 5–8μ in diameter, often with typical club-shaped swellings, conidiophores branched distally and slightly inflated terminally, with coronoid groups of 4–12 ovoid hyaline spores ranging in size from 7 to 25μ (mostly 15–19μ) long × 4–9μ (mostly 6–7μ) broad—*i.e.* very slightly longer and approximately the same breadth as in *B. anthophila* (Text-fig. 1). Kingma reports irregular spores similar to those of *B. anthophila*. Examination of a culture of *B. Trifolii* received from Dr Johanna Westerdijk, Central Bureau voor Schimmel-culturen, Baarn, Holland, showed that the two species did not differ in any essential features, although the Dutch culture had a more vigorous development of fluffy white aerial mycelium, with very infrequent conidiophores and fewer conidia per head. On peptone agar a similar frequently septate mycelium developed, which knotted into pseudo-sclerotia. These differences might be due to the age of the culture,

since in *B. anthophila* I have noticed some diminution in sporing propensity and increase in vegetative vigour after several subcultures.

It is significant that in this connection I was able to isolate a fungus which I regard as identical with *B. anthophila* from three-months-old red clover seed taken from infected plants, by washing the seed in sterile water and plating out the washings in agar. Moreover, when three-year-old samples of infected seed which had largely lost its germinability were sown on agar, *B. anthophila* developed extensively on dead seeds. Bondarzew centrifuged infected seed and found the conidia of *B. anthophila* present. There seems to be no clearly marked specific difference between the fungus which Kingma isolated from red clover seed and called *B. Trifolii* and that named *B. anthophila* by Bondarzew, which can also readily be isolated from seed. I am inclined to think that Kingma's fungus is probably a variety of Bondarzew's *B. anthophila*. It is uncertain if the spores of *B. anthophila* found on seeds can give rise to infection in seedlings, but from our present knowledge of the life history of the fungus this appears to be unlikely.

VI. Infection experiments

Bondarzew inoculated healthy plants by introducing a needle carrying conidia into the flowers, and observed the germination of the spores on the stigma and infection of the developing seed, but, as far as I am aware, there are no records of the examination of the anthers of progeny from such inoculated parents. Therefore a number of inoculations were performed by cross-pollination, with pollen contaminated with spores. The same procedure has been adopted for these infection experiments as is used for cross-pollination in routine work at this Station, namely, by the use of a strip of card about $1\frac{1}{2} \times \frac{1}{2}$ in., folded longitudinally, pointed at one end and roughened at the tip (Williams, 1925). The pollen of the progeny was examined in the following year. On the apparently healthy plants as many heads as possible were investigated, and several plants which were healthy in one year were re-examined the following year, in order to confirm the absence of infection. The experiment may be considered in two series (Tables I and II).

In these experiments approximately 50 per cent. of the resultant progeny were infected, whether the infection was derived from the male or female side. In the female plants, it is not possible to say whether the progeny became infected by the germination of conidia on the stigmas of the female parent, or by mycelium which entered the ovule directly from the axis below the ovary. No evidence of this latter path of entry was obtained in microtome sections of the young ovary.

Infection has also been induced by pollination with pollen derived

from a healthy plant, to which was added conidia from a monospore culture. Of twenty resultant plants, five showed infection, and from one of these the fungus was re-isolated and found to agree in characteristics with the original culture.

An attempt to induce infection from a subculture of *B. Trifolii* yielded negative results. Twenty-seven progeny plants were all healthy. However, in view of the age of the culture and the paucity of spores, this experiment cannot be considered as conclusive evidence that the species is not parasitic.

Table I. *Cross-infection*

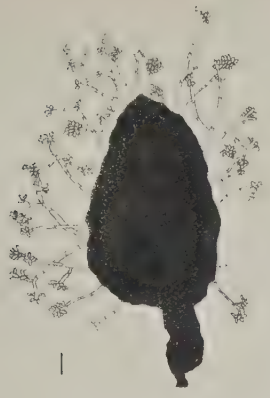
	(1) Cross-infection: Flowers on healthy plant pollinated with spores+pollen grains from infected plant			(2) Control: Flowers on healthy female parent used in (1) pollinated with pollen from healthy plant		
Plant No.	Total No. of progeny	No. of infected progeny	% infected	Total No. of progeny	No. of infected progeny	% infected
(1)	15	4	26·6	21	0	0
(2)	43	27	62·8	10	0	0
(3)	78	26	33·3	—	—	—
(4)	63	41	65·0	—	—	—
(5)	10	6	60·0	—	—	—
(6)	34	8	23·5	—	—	—
Totals	243	112	45·2	31	0	0

Table II. *Self-infection*

	(1) Self-infection: Infected female parent pollinated with pollen from healthy plant			(2) Control: Healthy female parent pollinated with pollen from healthy male parent used in (1)		
Plant No.	Total No. of progeny	No. of infected progeny	% infected	Total No. of progeny	No. of infected progeny	% infected
(7)	48	26	54·2	89	0	0
(8)	50	32	64·0	21	0	0
(9)	19	12	63·1	10	0	0
(10)	70	40	57·1	—	—	—
(11)	36	16	44·4	—	—	—
Totals	223	126	56·5	120	0	0

VII. Influence of the fungus on fertility

Although few actual figures are available there is definite evidence that the presence of this fungus has an important influence on fertility. It is evident from the previous section that an infected plant is not completely male sterile, although the number of fertile pollen grains must sometimes be very low. In an extreme example 384 florets "pollinated" with infected pollen yielded only 21 seeds (=5·5 per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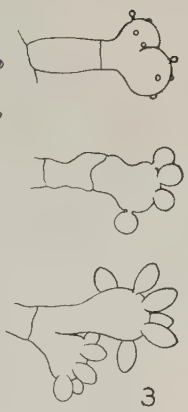
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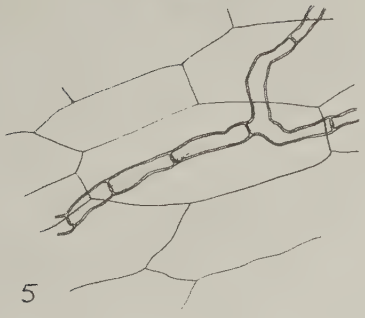
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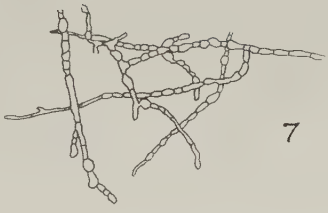
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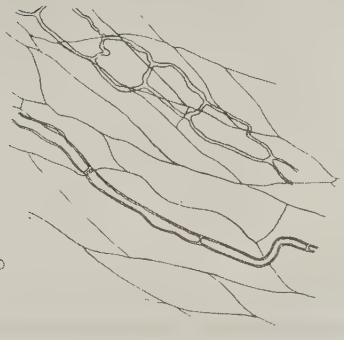
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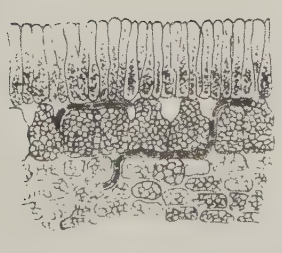
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cent.), whereas under normal conditions cross-pollination is expected to give 80–90 per cent. fertility.

Summary

Botrytis anthophila Bond. parasitises red clover, its spores partially replacing the pollen grains in the anthers of infected plants, and giving a characteristic ash-grey colour to them in place of the normal yellow of the pollen. The spores are disseminated by bees and, germinating with pollen grains on the stigmas of healthy plants, lead to the development of an intraseminal mycelium from which the systemic infection of the adult is derived. The disease has been shown to be widely distributed, and to have an adverse influence on fertility in red clover.

The fungus has been studied in pure culture and its pathogenicity proved by infection and re-isolation.

Botrytis antherarum Trifolii Schlecht is regarded as synonymous with *B. anthophila* Bond. It is probable that *B. Trifolii* Kingma, a fungus isolated from seeds of red clover, is a variety of the same species, differing perhaps slightly from the type in the size of conidia and in certain cultural characteristics.

I wish to record my thanks to Prof. Stapledon for providing the facilities for this investigation and for his interest in its progress. I am particularly grateful to Mr R. D. Williams, M.Sc., for providing the original fungal material, for placing his extensive collection of wild red clovers at my disposal for observations, and for his constant interest and advice. Special thanks are also due to Miss Kathleen Sampson, M.Sc., for her valuable advice and criticism, and to Miss R. M. Flemming, Librarian to the Anthropological Society, for translations of Russian papers.

EXPLANATION OF PLATE XVI

ALL DRAWINGS HAVE BEEN MADE WITH THE AID OF THE CAMERA LUCIDA

Fig. 1. Red clover anther infected with *Botrytis anthophila* Bond. Lateral view. × 40.
Fig. 2. Conidiophores and conidia of *B. anthophila* on red clover anther. × 500.
Fig. 3. Developmental stages of conidia: twelve-days-old culture on prune agar. × 500.
Fig. 4. Irregular spores (peptone agar). × 500.
Fig. 5. Hyphae in mesophyll cells of petal. Stained cotton blue lactophenol. × 500.
Fig. 6. Hyphae in base of filament. Stained cotton blue lactophenol. × 500.
Fig. 7. Hyphae showing barrel-shaped swellings (Dox's agar). × 150.
Fig. 8. Conidiophores and conidia of *B. Trifolii* Kingma (prune agar). × 500.
Fig. 9. *T.S.* Testa showing mycelium in subepidermal layers. Stained thionin-orange G. × 300.
Fig. 10. Surface view of testa in three focal planes: *a*, epidermis; *b*, mycelium in subepidermal intercellular spaces; *c*, subepidermal layers. Stained cotton blue lactophenol. × 300.

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ROTTING OF *GALTONIA* BULBS CAUSED BY *FUSARIUM CULMORUM* (W. G. SM.) SACC. AND *PENICILLIUM CORYMBIFERUM* WESTLING

By A. K. GHAMRAWY, B.Sc., Ph.D.

(With 1 Text-figure)

My attention was drawn to a storage trouble of *Galtonia* bulbs (*G. candicans* or Cape Hyacinth) by Dr G. H. Pethybridge, who had obtained diseased material from a Covent Garden warehouse. The bulbs were of Dutch origin, being imported in the late autumn, and in the present instance were intended for spring planting. Early in March a certain amount of wastage was noted among the stock, and it was at this period that the investigation was undertaken.

Diseased bulbs, on being cut open longitudinally, showed a rot progressing from the base upwards. The bases were wholly rotted and the infected parts of scales were likewise rotted and coloured dark brown. On the dead areas of some of the bulbs were abundant spores of a *Penicillium*, while on others a whitish mycelium was apparent round the base.

Isolations were made by sterilising the outside of diseased bulbs, opening up, and taking an inoculum from the junction of diseased with healthy tissue. From fifteen such isolations the following results were obtained:

From eight bulbs, a species of *Fusarium*,
From five bulbs, a species of *Penicillium*,
From two bulbs, bacteria.

The two fungi were each inoculated into fifteen bulbs of *Galtonia*. For this purpose a small incision was made at the place where the bulb scales join the basal plate, a piece of mycelium with spores inserted, and the wound sealed with a mixture in equal parts of vaseline and wax. On the opposite side of each bulb a similar hole was cut and then sealed up without inoculation so as to serve as a control. The inoculated bulbs were stored in equal batches at 15, 20 and 25° C. and examined after ten days. All became infected, more rapidly the higher the temperature, the control wounds remaining sound except in a few at 25° C. where they had obviously become invaded by the fungus advancing from the opposite side. At 25° C. the bases of some of the bulbs had wholly rotted within ten days. Re-isolations yielded pure cultures of both fungi.

The *Fusarium* from *Galtonia* bulbs was identified by Mr G. M. Wickens of the Plant Pathological Laboratory, Imperial College of Science, as a strain of *F. culmorum* (W. G. Sm.) Sacc., an identification

which was confirmed by Dr Wollenweber. A culture of the *Penicillium* was examined by Mr R. H. Bunting of the Imperial College Biological Field Station and found by him to agree in its cultural characters with *P. corymbiferum* Westling, though it was not compared with authenticated material of that fungus. For these identifications, the writer wishes here to record his thanks.

According to Thom (*The Penicillia*, p. 424, 1930), *P. corymbiferum* has been reported on a lily bulb which showed decay and masses of green spores between the scales. Recently Van Beyma (Abs. in *Rev. Appl. Mycol.* VIII (1929), 42) reports this fungus as causing a bulb disease of tulips in Holland.

The pathogenicity of the *Penicillium* and *Fusarium* isolated from *Galtonia* bulbs was compared with that of a number of similar organisms. Five bulbs of *Galtonia* were inoculated with each fungus in the manner described above, and left at laboratory temperature (15–18° C.) for five weeks, at the end of which time the weight of rotted tissue produced by each was determined (Table I).

Table I

Fungus	Av. weight of rotted tissue (gr.)
Penicillium corymbiferum (from *Galtonia*)	26·5
Fusarium culmorum (from *Galtonia*) ...	13·5
Penicillium sp. (laboratory contaminant)	0·5
Fusarium culmorum (from carnation) ...	3·1
F. bulbigenum (from *Narcissus*)	0·0
Controls	0·0

These results suggest that while some other species or strains of *Penicillium* and *Fusarium* may be able to attack *Galtonia* bulbs, they are much weaker in this respect than the two fungi isolated from this host.

A further test of the pathogenicity of these fungi on *Galtonia* bulbs was made by comparing the rate of invasion of scale tissue. The prepared scales were kept in moist Petri dishes and inoculated at the cut ends. The rate of attack could be determined approximately by noting the advance of the dark brown colour of the decayed tissue. The experiment was carried out at three temperatures, 15, 20 and 28° C. After twenty-three days the data shown in Table II were obtained.

Table II

Fungus	Av. linear advance of decay (cm.)		
	15° C.	20° C.	28° C.
Penicillium corymbiferum	1·2	2·0	0·7
Penicillium (from lily bulb*)	0·8	1·1	0·5
Penicillium (laboratory contaminant)	0·1	0·5	0·0
Fusarium culmorum (from *Galtonia*) ...	1·3	1·9	2·5
F. culmorum (from carnation) ...	0·7	0·7	0·9
F. bulbigenum	0·0	0·0	0·0
Controls	0·0	0·0	0·0

* Culture obtained from Dr Pethybridge, and identified by Mr Bunting as *Penicillium cyclopium* Westling.

Table II shows the same general features as Table I. It will be noticed also that *Penicillium corymbiferum* is much more active at 20 than at 28° C., whereas the reverse applies to the *Fusarium*. This effect of temperature on rate of invasion is in agreement with the growth curves of the two fungi on an artificial medium (2 per cent. malt agar). Fig. 1 shows that the optimum for growth of the *Fusarium* is definitely higher than that of the *Penicillium*.

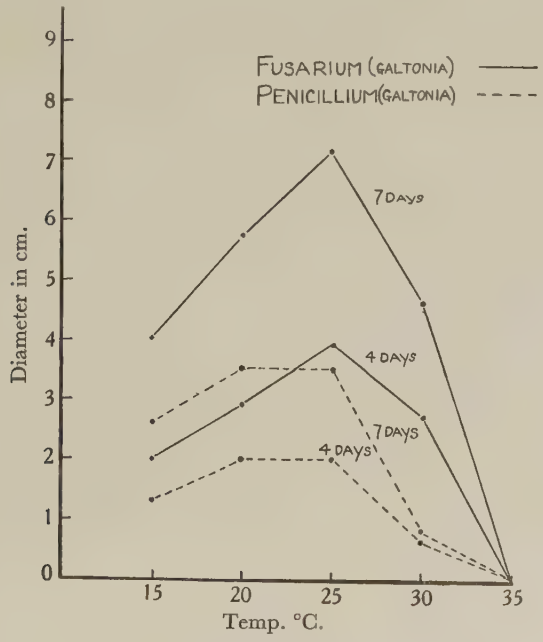

Fig. 1. Effect of temperature on growth of *Penicillium* and *Fusarium* from *Galtonia* bulbs.

The pathogenicity of the various fungi to *Galtonia* bulbs in the soil was also tested. Five bulbs were inoculated with each fungus in the manner already described and immediately planted in pots of ordinary garden soil in the open. The experiment was begun in the first week of May. Table III records the appearance of the plants in the middle of August, *i.e.* fully three months later.

Table III

Fungus	Full-grown flowering plants	Dwarfed, non-flowering plants	Blanks
Penicillium corymbiferum	1	3	1
Penicillium (from lily)	3	0	2
Penicillium (laboratory contaminant) ...	4	1	0
Fusarium culmorum (from *Galtonia*) ...	5	0	0
F. culmorum (from carnation)	5	0	0
F. bulbigenum	5	0	0
Controls (wounded but not inoculated)	5	0	0

Thus, under the conditions prevailing in the soil during this experiment, the most active fungi appeared to be the two Penicillia isolated from bulbs.

Subsequent examination of the weak plants showed that the main bulb had been partially destroyed but that a number of young daughter bulbs had formed on the side still remaining healthy. When the bulbs that had failed to come up were examined, they were found to be completely rotted.

A parallel series of bulbs was inoculated by dipping in spore suspensions of the various fungi and planted as before. All these bulbs grew normally, thus indicating that the active fungi are wound parasites only.

Summary

1. A strain of *Fusarium culmorum* (W. G. Sm.) Sacc. and *Penicillium corymbiferum* Westling, which were isolated from rotting *Galtonia* bulbs, were found to be actively pathogenic to the bulbs under a variety of storage conditions.

2. The *Fusarium* is the more active parasite at rather high temperatures (28° C.).

3. Up to the present only the *Penicillium* and a similar form isolated from lily bulbs have been found to cause rotting of bulbs planted in soil in the open.

TREMELLA MYCETOPHILA PECK

By J. RAMSBOTTOM

(With Plates XVII and XVIII)

Teratological specimens have always an interest. It would probably be no exaggeration to say that they are as common in fungi as in flowering plants, though there have been fewer attempts to explain them.

During the Autumn Foray (Hurt Hill Wood, September 20th, 1932), Mr P. O'Connor handed me three specimens of *Collybia* (*Marasmius*) *dryophila* bearing outgrowths. Mr Somerville Hastings kindly photographed them and they were on exhibit during the meeting, being put in preserving fluid for that purpose.

The main interest in these outgrowths is that when first recorded by C. H. Peck in 1879 they were mistaken for a parasite and named *Tremella mycetophila*. "Suborbicular, depressed, gyrose-plicate, tremelloid-fleshy. Slightly pruinose, yellowish or pallid, 4''–8'' broad. Stem and pileus of *Agaricus dryophilus*." G. F. Atkinson (1900), E. A. Burt (1901) and D. S. Hone (1909) followed him in regarding it as a parasite though Burt transferred the "fungus" to *Exobasidium* because the substance was not gelatinous nor the basidia cruciately divided. In 1915, however, Burt no longer regarded it as a parasite "but, rather, as a teratological production of *C. dryophila*, induced by protracted wet weather during development of the fructification." Boudier in his last mycological paper (1917) describes and draws *Exobasidium mycetophilum*, but says "elle pourrait bien n'être à mon avis qu'une modification produite par le développement de conidies, comme on le remarque souvent chez divers champignons surtout Polypores, Fistulines et autres." C. G. Lloyd (1917) also gives a photograph.

A. H. R. Buller (1924) in summarising the accounts of the abnormality says he knows of no record for this country, and in opposing Burt's hypothesis of its production states that "the excrescences occur in Manitoba but not in England, which is the opposite of what we should expect" if it were correct.

The present examples, however, are not the first I have seen in this country. So far as I remember the first were at the Epping Foray in 1915—when I did not realise their interest; others I saw at Windsor Park in company with Mr W. D. Buckley in 1921.

It is difficult to explain why the growths should have given so much trouble in determination. Peck was obviously misled by their appearance. He gave the structure a specific name and later it was

taken on trust as an entity. Whether or not, the fact remains that other agarics occasionally have not dissimilar outgrowths, *e.g. Collybia butyracea*, *Laccaria laccata*. Moreover, the fungus figured by Miss Hone and reproduced by Buller as his Fig. 193 does not appear to be *Collybia dryophila*: it is not the same fungus as is shown in Fig. 192 and it differs in its basidia and spores. E. Ulbrich (1926, 1928) lists such malformities as "Mutationen" which seems an unnecessary complication.

It is certain that if we regard *Tremella mycetophila* as a distinct fungus we shall be driven to describe a whole series of similar parasites.

As there seemed to be still some uncertainty about the matter the preserved material was sectioned by Miss F. L. Stephens to whom I am also indebted for assistance in the examination of sections. The smaller outgrowths and most parts of the larger ones were solid, but these in their thickest portions were slightly hollow. There was nothing approaching the hollow structures shown on the stipe in Buller's schematic figure (Fig. 194 *c*, *d*, *e*). The larger specimens were much convoluted but not collapsed; indeed it would appear that really hollow structures with merely a spore-bearing layer would be morphologically impossible.

The first point noticeable in stained microtome sections is that the outgrowths take up and retain all stains much more strongly than do the pileus and gills with the exception of the hymenium. It is reasonable to suppose that this is on account of their rapid growth and the abundant food material present. The cuticle of the pileus is thin and the outgrowth arises from a small area and is not attached throughout its length. Apparently as growth proceeds there is coalescence between outgrowths. The area of attachment on the stipe is also restricted—so much so that one has to be careful not to knock them off.

The outgrowths are composed of a closely interwoven mass of hyphae which vary from 4 to 8μ in width, those of the portion adjoining the pileus being on the whole wider. Owing to its avidity for stains this tissue at first sight appears to be entirely different from the parent tissue; in fact a superficial examination would incline one to regard it as parasite. The tissue of the pileus is composed of loosely woven hyphae 7–14μ wide. When an outgrowth breaks away it brings a little of the pileus tissue with it and it is then easy to see that the hyphae are continuous. For some reason or other, about which it is useless to guess*, a period of renewed growth of hyphae at the surface of the pileus (and stipe) has occurred after normal growth has ceased, and an overgrowth has formed.

Burt in his account of the anatomy of *Exobasidium mycetophilum* states

* Occasionally what is apparently a dipterous insect is to be seen in a cavity. (See Pl. XVII, fig. 2.)

that "innumerable minute conidial spores [about $2 \times 1\frac{1}{2}\mu$] occupy the spaces between the hyphae," and that these "are seen to be formed in bead-like chains by the constriction of the smaller hyphal branches." Buller has reproduced Burt's figures and Ulbrich (1926, 1928) has copied them from Buller and apparently also in his revision of Lindau's *Die Höheren Pilze* where he says (p. 323 footnote), "Eine konidienbildende 'tremelloide' Form ist von dieser Art [*Collybia dryophila*] mehrfach in Deutschland (b. Berlin, Hamburg, Siegen, Darmstadt) beobachtet. Der Hut (selten auch der Stiel) zeigt sehr auffällige gekröseartige Gewebebildungen mit etwa 1μ gr. Konidien."

Miss Hone does not describe conidia.

Boudier in his description has "conidiae minutae, rotundatae, $1-2\mu$ crassae, numerosae."

It was a matter of great surprise not to be able to find any trace of conidia in the numerous sections of the present specimens. Some of the hyphae are cut across and these look like conidia, but neither their arrangement nor their size warrants the assumption that they may have been misinterpreted. Such internal conidia are very unusual in structures of this kind which have a greater consistency than the normal fungus and probably lie on the ground long after the pileus has decayed: if the overgrowths have any biological significance and are not merely teratological one would expect to find chlamydospores.

The whole of the surface of the overgrowth is covered with a hymenium. As the photomicrograph (Pl. XVII, fig. 2) shows this hymenium extends right up to the cuticle of the pileus. The presence of basidia in addition to a crop of conidia apparently led Burt to consider whether the "fungus" was entitled to special generic rank. He decided to transfer it to the Thelephoraceae "provided the distortions are not merely monstrous outgrowths of *Collybia dryophila* itself—which I do not believe after microscopic comparisons with the lamellae of *C. dryophila*." Unfortunately my specimens were badly fixed and details are difficult to make out. The hymenium on the gills has a well-marked subhymenial layer and a definite trama. The basidia measure $19-21 \times 3{\cdot}5\mu$ and the spores $4-6 \times 3-4\mu$: the trama cells are $5-10\mu$ wide. The hymenium on the surface of the overgrowth has no subhymenial layer proper but the general lie of the hyphae is parallel to the surface. The basidia measure $15-17 \times 3-7\mu$ and the spores $3-5 \times 1-3\mu$.

There can be no doubt that *Tremella mycetophila* Peck is merely an overgrowth on the pileus and stipe of *Collybia dryophila*. It will be interesting to see whether any future examples found in this country have the chains of conidia so clearly figured by Burt.

I have to thank Mr E. H. Ellis for staining and photographing some sections.

EXPLANATION OF PLATES XVII, XVIII

Plate XVII

Fig. 1. *Collybia dryophila* showing tremelloid outgrowths.
Fig. 2. Median longitudinal section through cap and tremelloid portion.

Plate XVIII

Fig. 1. Transverse section of normal gills showing hymenium and subhymenium. × 45.
Fig. 2. Basidium and spores from normal gill. × 900.
Fig. 3. Basidia on tremelloid hymenium. × 900.
Fig. 4. Hymenium and subhymenium of tremelloid growth (right) showing junction with normal surface of cap (left). × 45.

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Fig. 1

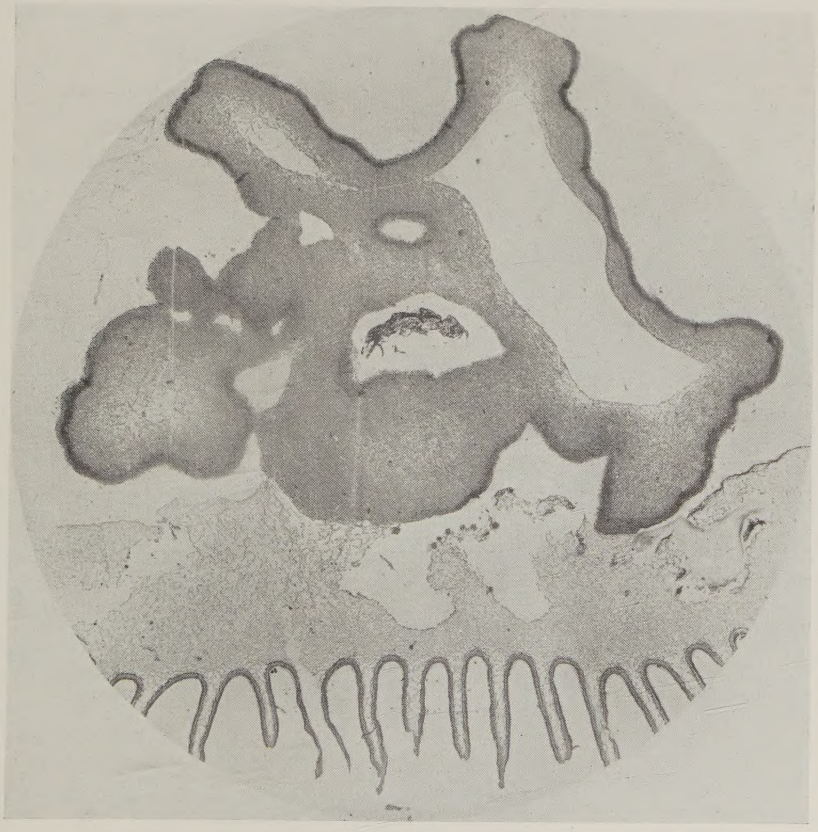

Fig. 2

TREMELLA MYCETOPHILA PECK

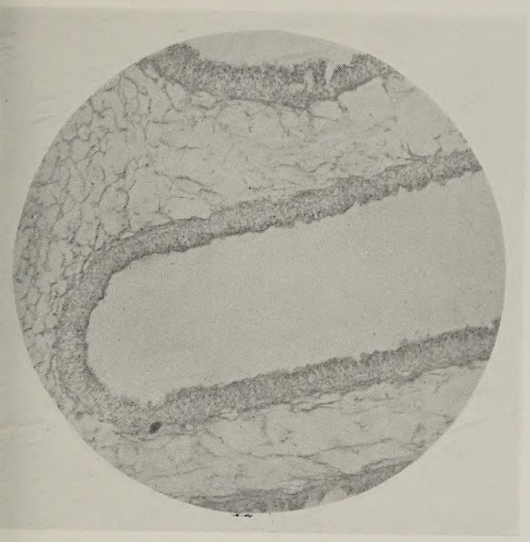

Fig. 1

Fig. 2

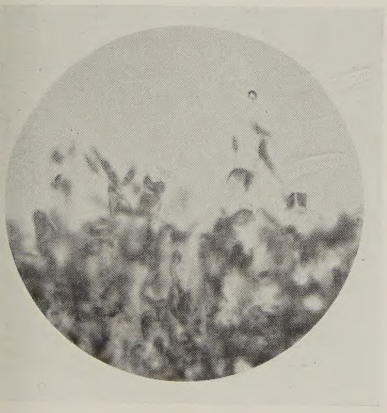

Fig. 3

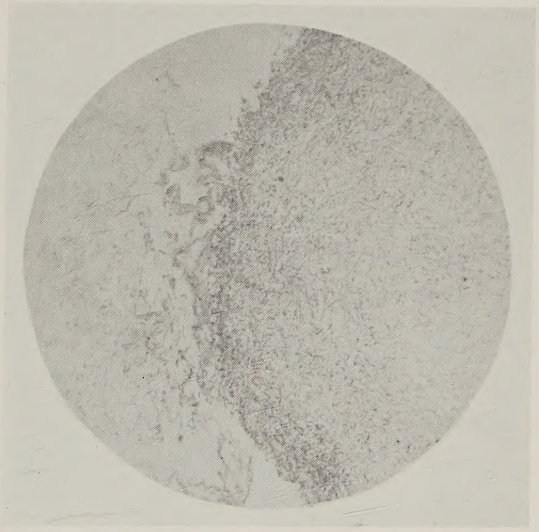

Fig. 4

TREMELLA MYCETOPHILA PECK